THE SEPSIS SYNDROME AND THE "ONE SIZE FITS ALL" CONSTRUCT: THE EMPEROR HAS NO CLOTHES!!

by

Hernando Gomez

Specialist in Critical Care Medicine, University of Pittsburgh, 2010

Specialist in Anesthesiology, Universidad Nacional de Colombia, Colombia, 2007

Doctor in Medicine and Surgery, Universidad Nacional de Colombia, Colombia, 2001

Submitted to the Graduate Faculty of

the Multidisciplinary MPH Program

Graduate School of Public Health in partial fulfillment

of the requirements for the degree of

Master of Public Health

University of Pittsburgh

2014

UNIVERSITY OF PITTSBURGH

GRADUATE SCHOOL OF PUBLIC HEALTH

This essay is submitted

by

Hernando Gomez

on

August 7, 2014

and approved by

Essay Advisor:
David Finegold, MD
Director, Multidisciplinary MPH Program
Graduate School of Public Health
University of Pittsburgh

_____

Essay Readers:

Lisa Weissfeld, PhD
Professor, Biostatistics
Graduate School of Public Health
University of Pittsburgh

_____

John A. Kellum, MD
Professor, Critical Care Medicine
School of Medicine
University of Pittsburgh

_____

Michael R. Pinsky, MD
Professor, Critical Care Medicine
School of Medicine
University of Pittsburgh

_____

David Finegold, MD

THE SEPSIS SYNDROME AND THE "ONE SIZE FITS ALL" CONSTRUCT: "THE EMPEROR HAS NO CLOTHES!!"

Hernando Gomez, MPH

University of Pittsburgh, 2014

ABSTRACT

Background: Sepsis, a syndrome characterized by a systemic, (often overwhelming) inflammatory response to infection is an under recognized, potentially lethal, public health problem in developed and developing countries. Furthermore, is unlikely that it will improve, as other than standard critical care support, there are no effective specific treatment strategies. Most of the therapeutic trials conducted in the last four decades, other than the lack of benefit, have consistently shown that subgroups of septic patients respond differently to the same treatment. This has lead to the thought that sepsis may not be a unique syndrome only differentiated by grades of severity, but rather a syndrome that encompasses diverse phenotypes that behave differently and thus may respond different not only to injury but also to treatment. Thus, the aim of this study is to explore if distinct phenotypes exist in a cohort of critically ill patients with suspected sepsis, and if these can be identified through clinical available data. This is highly relevant to the public health aspect of Sepsis, as it challenges the current paradigm, and provides the basis to develop a new approach that may lead finally to an effective reduction of morbidity and mortality. Methods: We used a large database of critically ill patients (HiDenIC-8). We selected a population of patients with "suspected sepsis" defined as having blood cultures sent or being started on antibiotics within 24 hours of admission to the ICU. We defined demographic, clinical and available laboratory variables to include in the clustering algorithm, and selected

them on the basis of availability, and absence of redundancy. We used hierarchical clustering to evaluate the possible number of clusters according to the data structure, and then ran K-means method to determine the actual cluster schedule. Results: We found 13 clusters, 8 of which included more than 70 subjects (~2.5% of entire population). We found important differences in demographic, clinical and laboratory data at admission, and also, different clinical trajectories in terms of patterns of organ dysfunction and mortality. Conclusion: The present study has demonstrated that an unsupervised clustering technique based on frequently collected demographic, clinical and physiologic data can be used to derive distinct, biologically sound clusters of patients who clinically behave differently from each other.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEGEMENTS

I would like to dedicate this work to three very important people in my life. To my wife Silvia, my best friend, my life companion, my confident, my present, my future, and all of my reasons. Thank you for filling my life with unthinkable happiness, for showing me where home is, and for supporting me on every adventure I embark on.

To my parents Nancy and Alonso, the first heroes I have recollection of, my guide and example. To them, who taught and loved me more than I could ever hope to express with words. Thank you mom for showing me what courage, generosity and compassion really mean, for teaching me the importance of respect and caring for others, of helping without expecting retribution, of sharing, of always having one more place at our table, of embracing family, of what love really means. Thank you for laughing and crying with me, for your beautiful life and for sharing it with me. Thank you dad for patiently showing me the ways of life by example, for teaching me to believe in myself, to trust hard work and knowledge as the only real meaningful assets anyone can ever have, to always speak the truth, and to look at adversity in the eye regardless of the consequences; thank you for teaching me that the right path is almost never the one of least resistance, and that success is not measured in paper or gold, but in the eyes of those who we care for. I can only hope that one day I can be as good as a parent as you both have been to me.

To my mentors, Doctors David Finegold, Lisa Weissfeld, John Kellum and Michael Pinsky, thank you for your support, willingness, patience, time and commitment to my career. To Dr. Lisa Weisfeld, who took me as a mentee without hesitancy, and gave me much, much more than I would have ever expected. To Doctors Kellum and Pinsky, thank you for believing in me, for taking me under your wing, and for lending me all your experience and wisdom. A very special thanks to Dr. John Kellum, without whom this initiative would have never taken off the ground; thank you for you time, your trust and your will to help me become a better physician scientist.

Last, but certainly not least, very special thanks to Florentina Sileanu who helped me acquire the database that made this effort possible. Thank you for your kindness, for your patience with so many silly questions, and your willingness and disposition to help develop this project.

1.0 INTRODUCTION

Sepsis, a syndrome characterized by a systemic, overwhelming response to infection continues to be the leading cause of death in the critically ill patient. It is a lethal, incapacitating syndrome, which represents an important burden to the health care system, and has become a major health care problem.[1,2] Importantly, as it usually develops in the hospital, it has largely been under recognized as a public health problem in the community[3], and currently, worldwide campaigns aim to solve that by making the public aware of its existence and its lethality.[4]

The definition of sepsis has evolved over time, and has done so intimately related to the evolution of our own understanding of the syndrome. The term sepsis is attributed to Hippocrates, and was used to represent one of the two processes by which biologic material was broken down. Indeed Sepsis or "a process by which flesh rots" was associated with bad smell, and putrefaction, and considered medically dangerous, whereas Pepsis was a controlled, helpful process, analogous to fermentation or digestion.[5] Two millennia after the Greco-Roman era, the advent and consolidation of the germ theory changed the perspective of the syndrome, attributing its consequences to the impact of an external agent (i.e. a bacteria) on the body, and opening new therapeutic avenues. However, the germ theory did not fully explain the entire clinical syndrome, and it was recognized that the host's response to infection was a key feature and the potential driver of the process. Consequently in 1992, a group of investigators lead by Dr. Roger Bone put

together a collection of signs and symptoms to define the "sepsis syndrome" as entry criteria to study the effects of methylprednisolone on this devastating condition. This was later defined as the Systemic Inflammatory Response Syndrome or SIRS. Thereafter, iterations of these consensus conferences have moved forward the definitions of sepsis and categorized the different stages or severity of the syndrome. Accordingly today, sepsis complicated with organ dysfunction or hypoperfusion was defined as *Severe Sepsis*, and complicated with hypotension refractory to fluid resuscitation, as *Septic Shock*[4]. These definitions have been fundamental to standardize the language throughout the scientific, clinical and research communities, and has allowed to test and implement several diagnostic and therapeutic strategies. However, daily practice continues to reveal that in many cases these definitions are still incomplete, too broad, and some times even inadequate to predict outcome, but even more importantly, to establish specific therapy. This is strongly supported by the fact that to date, and after thousands of patients and millions of research dollars spent, there is no one single effective specific treatment for sepsis other than the use of antibiotics, fluid and vasopressor resuscitation and organ support provided in the intensive care units. Furthermore, it has been recognized in many of these "failed" trials that some of these therapies (i.e. Activated protein C), may seem beneficial to certain patients, suggesting again that the "one size fits all" approach is far from adequate, that there must be important clinical and subclinical differences in how sepsis presents that may open avenues to specific treatments and that the approach to investigating Sepsis must change[6]. This construct is supported in the pediatric literature, where differential phenotypes of sepsis have been defined and studies are underway to determine whether or not they will serve as primers for the use of specific therapies.[7] Thus, it is imperative that these different phenotypes are sought, found, described and studied, so that treatment strategies can be designed and tailored to

populations that will respond to them, and will, for the first time in half a century, impact the mortality rate of this lethal, morbid and expensive syndrome.

In accordance with the above, the aim of this study is to determine if there are different patterns of sepsis in a large population of septic critically ill patients, and furthermore, whether or not these patterns are associated with different patterns of organ involvement and recovery, and with patient oriented outcomes. We have designed this study with the assumption that we do not know how these phenotypes look like or what defines them. Instead, have used an unsupervised clustering analysis approach to retrospectively interrogate a large prospectively collected data base, that will allow for natural grouping of patients around specific variables available in this database, but also, commonly assessed in any given ICU.

1.1 SIGNIFICANCE

The majority of the clinical trials conducted in the last twenty years looking for specific therapies for sepsis have failed to demonstrate any sustainable benefit on mortality.[8] Although there may several reasons for this frustrating lack of success, a common feature stands across all of these studies. As a result of the preconception that sepsis is indeed one disease, the inclusion criteria of these trials has been based mainly on the definition of the sepsis syndrome, and only varying according to the severity of the condition. For instance, the PROWESS trial[9] randomized patients with known or suspected infection, plus three or more signs of systemic inflammation and sepsis-induced organ dysfunction of at least one organ or system. In current definitions, these

would be patients with severe sepsis. Despite the lack of encouraging results, these trials have greatly informed the understanding of the disease. Specifically, most of these trials have demonstrated that the effects of different therapeutic strategies are not the same in all patients, and that in certain subgroups any given agent may be helpful or harmful.[10] This is important because it reflects on the fact that perhaps the original conception of sepsis being one disease may be insufficient, and that there may be different phenotypes within the syndrome, that will respond differently to the same intervention. This is a fundamental step to take as understanding whether or not different phenotypes exist will potentially redefine the syndrome, change the pre-established pathophysiological paradigm and most importantly, will modify our approach to treating this deadly condition.

1.2 SPECIFIC AIMS AND HYPOTHESES

Our overarching hypothesis is that in a cohort of critically ill patients with "suspected sepsis" different clinical phenotypes can be identified based on demographic, clinical, physiologic and common laboratory data. We will develop this hypothesis in the setting of the following specific aims:

- Specific Aim 1. To identify naturally occurring phenotypes in a cohort of critically ill patients with suspected sepsis using unsupervised clustering analysis.
    - *Hypothesis 1.1.* Unsupervised cluster analysis will identify distinct phenotypes of patients on the basis of usual demographic, clinical, physiologic and laboratory data.

- Specific Aim 2. To assess the association of identified clusters with measures of patient oriented outcome.
    - *Hypothesis 2.1.* There will be differences in 90 day and 1 year mortality between clusters.
    - *Hypothesis 2.2*. There will be differences in hospital and ICU length of stay between clusters.
    - *Hypothesis 2.3*. Clusters will have different patterns of organ/system compromise.

2.0 METHODS

The study was conducted in accordance with institutional review board guidelines and approval.

2.1.1 Source population

We used The High-Density Intensive Care (HiDenIC-8) database for this study. This dataset includes clinical, laboratory and demographic information on 45,655 adult patients admitted to a tertiary care institution (Presbyterian University Hospital-UPMC, Pittsburgh, PA), to any of eight intensive care units (ICU: cardiac, transplant, neurological, trauma, surgical and medical) in a period of eight years (from July 2000 to October 2008). From this dataset, we selected a population of patients based on the following inclusion criteria: 1. Age > 18 years; 2. Patients admitted to the ICU with criteria for "suspected sepsis". Suspected sepsis was defined as having blood cultures sent or being started on antibiotics within 24 hours of admission to the ICU. We used the following exclusion criteria: 1. Patients who developed sepsis or reached the "suspected sepsis" criteria beyond 24 hours after admission to the ICU; 2. Patients with ICU length of stay of less than 48 hours; 3. Patients who died within the first 48 hours after ICU admission. The cohort selection process and final study population is shown in Figure 1.

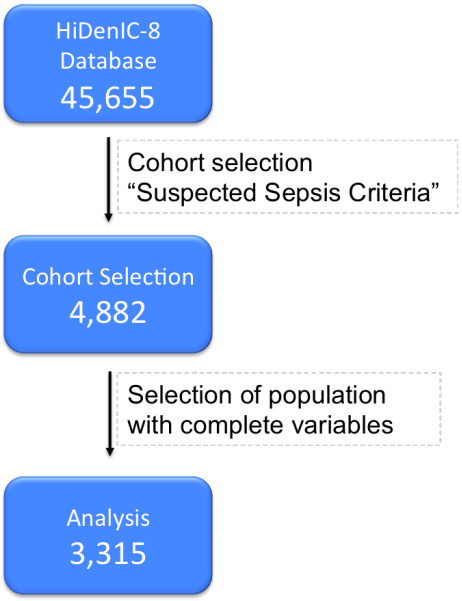


Figure 1. Consort Diagram

2.1.2 Data base characteristics, data collection and manipulation of the database

The HiDenIC-8 database merges information on all patients admitted to the ICU in the period from 2000-2008, from several sources including the electronic health record, the Medical Archival Repository System (MARS) which contains MARS-MediPAC (demographic, clinical, insurance, and diagnostic related group information), MARS-Financial (itemized billing data) and MARS-Clinical (laboratory data, International Classification Diseases, 9th edition or ICD-9, surgical procedures, and various other elements like reports, clinical notes and discharge summaries). All the MARS data was combined with the Eclypsis database which holds all the clinical information and the inpatient clinical record. Finally, information from the United States Renal Data System (USRDS) and the National Death Index (NDI) was obtained by the honest broker and was merged with HiDenIC-8 database before de-identification.

In order to optimize the clustering analysis, we selected a cohort of patients that would have all the variables of interest. We excluded the patients that did not have a complete set of variables for the clustering analysis, obtaining a final cohort of 3315 patients. Comorbidities were quantified by calculating the Charlson-Deyo index[11,12] as detailed in Appendix A1.

2.1.3 Hierarchical clustering and variable selection

The aim of the present study is to identify possible naturally occurring subgroups or phenotypes in a large database of patients with suspected sepsis. Clustering analysis is a methodology that allows exploring the existence of such natural groups within any given data set. In addition, we used an unsupervised approach, in which we allowed the clustering algorithm to determine the structure of the data based on the data set and not on pre-specified characteristics. We chose this approach because we based our analysis on the assumption that we were oblivious to how these clusters should look like, or to how many should there be in the sampled cohort. Although we assigned the variables that the model would use for clustering, we were careful to select them on the basis of objective criteria. We examined all the non-date variables available in the data set, and established redundancy as the primary exclusion criteria for variable selection. Redundancy was established through a correlative matrix where significant correlations between variables were identified. When present, if biologically plausible, the variables were assumed to carry similar information (redundant), and thus, one of the two variables was selected and used in the clustering analysis. Eighteen variables were selected using this methodology and are listed in table 1.

Table 1. Variables used for clustering analysis

Variables	
Time to sepsis	Hemoglobin
Baseline Creatinine	Platelet count
APACHE III score	White blood cell count
Age	Arterial O_2 saturation
Weight	Temperature
Systolic arterial pressure	Lactate
Diastolic arterial pressure	Comorbidities
Mean arterial pressure	Transplantation

Note: All variables used at this stage are variables obtained as first value upon admission to the ICU.

We used two methods of clustering. First, we used Hierarchical clustering to determine the number of clusters occurring naturally in the dataset. We used clustering around the centroid, using the squared Euclidean distance, and then plotted the distance coefficients to identify the "step-off" in a scree diagram. A scree diagram or plot is a graphical display of the variance of each component in a dataset and is used to define the amount of components that explain the majority of the variation in the data. We identified this "step-off" at the observation 3302. The number of clusters was defined by the difference between the total number of observations and the "step-off": 3315 - 3302 = 13. We then partitioned the data using the K-means method with the number of clusters derived from the Hierarchical clustering analysis (13 clusters). We

determined a threshold "n" below which a cluster would be excluded. Such threshold was 2% of the total population, or n = 66.

2.1.4 Characterization of clusters

We describe the characteristics of each cluster in three domains: 1. Demographics; 2. Clinical/Physiologic data at admission; and 3. Process of care within 24 hours of admission. This description will provide the information to inform and develop Specific Aim 1. At a later stage, not contained in this manuscript, this information will serve as preliminary data to test these phenotypes in a different cohort of patients and determine whether or not these clusters will perform in a similar manner in terms of organ dysfunction patterns and mortality. In addition, we will expand the amount of data at ICU admission to biologic markers and microcirculatory data to evaluate whether these markers can predict which cluster is most likely to fit each patient. Finally, we intend to understand the biology of each cluster in animal models, in order to formulate possible subgroups of patients that would benefit from different types of treatment.

2.1.5 Measures of outcome

We evaluated the association of each cluster with measures of outcome. Primary endpoints were mortality at 90 and 365 days. Secondary outcome measures were ICU and hospital length of stay, and organ system involvement at 72 hours and 30 days. We evaluated organ system involvement as shown in table 2. One of the objectives of this study was to evaluate if different clusters would be associated with different patterns of organ/system involvement, and we explored this in a descriptive way. To determine whether or not organ dysfunction was present

we assessed each of the variables listed in table 2 according to pre-specified rules based on well known organ function assessment scores (Sequential Organ Function Assessment Score or SOFA[13]. These pre-defined rules can be found in Appendix A2. As shown in table 2, each organ was assessed by two distinct variables (except for hematologic system). As a general rule, organ compromise was confirmed when any one of the variables assessing a specific organ, was found to be abnormal in the moderate to severe range. For instance, lactate levels, used to evaluate cardiovascular function, were considered altered when values were higher than 2 mmol/L. a mild alteration was considered to be a lactate level between 2-3, a moderate 3-4, and a severe alteration > 4 mmol/L. Thus the cardiovascular system was said to be dysfunctional when lactate levels were above 3 mmol/L in the moderate to severe range. Further information on this assignment rule is contained in Appendix A2.

Differences between clusters in categorical or binomial variables were assessed using Pearson's chi2 test. Differences in continuous variables between clusters were assessed using either one-way analysis of variance (when normal distribution criteria was satisfied), or Kruskal-Wallis equality of populations rank test (when absent normal distribution was found).

Table 2. Evaluation of organ/system compromise

Organ/system involved	**Variable**	**Measured at:**
Cardiovascular	Lactate	72 h
	Use of vasopressors	72 h and 30 days
Pulmonary	PaO2/FiO2	72 h and 30 days
	Need for mechanical ventilation	72 h and 30 days
Renal	Creatinine	72 h and 30 days
	Change in Creatinine	72 h and 30 days
	Need for RRT	Within 24 – 72 h and any time between 72h and 30 days
Hematologic	Platelet count	72 h and 30 days
Hepatobiliary	INR	72 h and 30 days
	Total Bilirrubin	72 h and 30 days

3.0 RESULTS

3.1 CLUSTERING ANALYSIS

3.1.1 Hierarchical clustering, Scree plot and K-means

The clustering analysis was performed using a two-step approach. First a Hierarchical clustering method was used to identify relatively homogeneous groups (clusters) of cases based on the selected variables. The Hierarchical clustering analysis and the scree plot suggested the data could be divided into 13 clusters as shown in Figure 2.

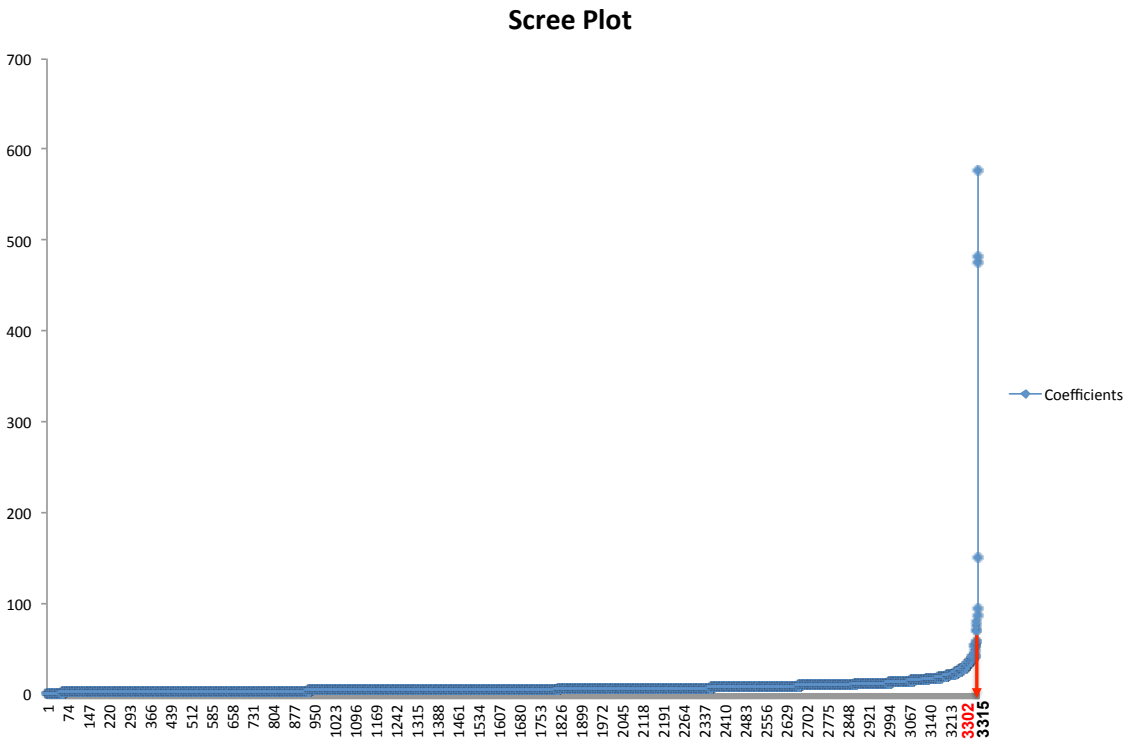


Figure 2. Scree Plot.

The K-means analysis was ran using these possible 13 clusters and obtained a partitioning of the cohort as shown in table 3. However, based on our pre-specified threshold, we excluded clusters 3 (n=28), 6 (n=2), 8 (n=2), 9 (2), and 10 (37) from the analysis, thus reducing the number of effective clusters to 8. Only data on these 8 clusters is reported here forth unless stated otherwise.

Table 3. Results of K-means analysis and summary of the 13 clusters.

Cluster	n	Percent (%)	Cumulative frequency (%)
1	413	12.46	12.46
2	525	15.84	28.30
3	28	0.84	29.14

Table 3 Continued

4	208	6.27	35.41
5	657	19.82	55.23
6	2	0.06	55.29
7	73	2.20	57.50
8	2	0.06	57.56
9	2	0.06	57.62
10	37	1.12	58.73
11	762	22.99	81.72
12	417	12.58	94.30
13	189	5.70	100

3.1.2 Description of patient characteristics in the entire cohort

The analysis was done on a cohort of 3315 patients with "suspected sepsis". The general description of the entire cohort is shown in table 4 below. However, this description is on 3244 patients given that the patients allocated to clusters 3, 6, 8, 9, and 10 have been excluded.

Table 4. Descriptive statistics of the entire cohort: demographics and admission to ICU data including physiologic, risk stratification (APACHE score), DRG classification, and comorbidities.

Variable		Mean	Standard deviation	Min	Max
Demographic data					
Age (years)		58.3	16.7	19	106
Weight (Kg)		83.3	26.9	21	294.8
Number of Comorbidities		0.96	1.67	0	13
Gender (n/%)	Female	1480	45.6		
	Male	1764	54.4		
Number of transplants (kidney and/or liver)		0.10	0.35	0	2
Clinical/Physiologic					
APACHE III		88.9	14.7	46	148
SBP (mmHg)		123	30.2	40	263
DBP (mmHg)		64	19.9	11	202
MAP (mmHg)		83	21.4	22	215
Temperature (^{o}C)		36.9	1.5	30	41
DRG (n/%)	Medical	1678	51.7		
	Surgical	1369	42.2		
	Missing	197	6.1		
Time to sepsis (minutes)*		251	304	0	1440

Table 4 Continued

Lactate (n=2117) (mmol/L)	3.5	3.7	0.3	31
Base deficit (mEq/L)	2.9	6.9	-25	30
pH	7.35	0.12	6.6	7.69
$PaCO_2$ (mmHg)	40	13.3	7	167
PaO_2 (mmHg)	141	89.9	19	540
SaO_2 (%)	96	7.7	0	100
Hemoglobin (mg/dL)	10.9	2.3	3	20.9
White blood cell count ($1x10^9$/L)	13.9	9.3	0.4	106
Platelet count ($1x10^9$/L)	207	122	2	971
Glucose (mg/dL)	158	70	14	651
Creatinine (mg/dL)	1.1	0.89	0.1	21
Process of care				
Vasopressor use** (n/%)	1394	42.9		
Mechanical ventilation** (n/%)	2248	69.3		
RRT ** (n/%)	40	1.2		
FiO_2	0.57	0.32	0	1.0
Fluids administered*** within first 24 h (ml)	4240	2933	3	16382

RRT=Renal replacement therapy; *=Time from admission to establishment of diagnosis of "suspected sepsis"; **=at admission; ***=within first 24 hours after admission; FiO_2 = Inspired fraction of Oxygen

3.1.3 Description of patient characteristics per cluster

The analysis of the characteristics of the population in each cluster will be displayed in three domains as stipulated in the methods section. These domains will be: 1. Demographics; 2. Clinical/Physiologic data at admission; and 3. Process of care within 24 hours of admission. Table 5 summarizes these characteristics per cluster, including outcome data.

Table 5. Demographic, clinical/physiologic, process of care and outcome data per cluster

Variable	**Cluster**							
	1	**2**	**4**	**5**	**7**	**11**	**12**	**13**
n	413	525	208	657	73	762	417	189
Demographic data								
**Age (years)	60.9±17.0	58.4±17.0	56.2±14.7	60.8±16.7	53.4±17.4	57.1±16.2	56.0±15.9	56.9±17.8
Weight (Kg)	81±25	82±26	85.6±29.6	84.2±26.6	80.7±16.9	84.7±28.1	84.4±28.0	79.3±26.0
Gender: Females (n/%)	209/50.1	233/44.4	93/44.7	319/48.6	22/30.1	333/43.7	183/43.9	88/46.6
Mean Charlson-Deyo score	1.0±1.7	0.8±1.5	0.9±1.6	1.2±1.9	0.8±1.7	0.9±1.6	0.9±1.7	0.8±1.4
Number of transplants (kidney and/or liver)	0.09±0.31	0.06±0.26	0.10±0.36	0.12±0.38	0.06±0.28	0.05±0.28	0.14±0.40	0.13±0.39
Clinical/Physiologic								
**APACHE III	81±11	81±10	102±15	88±14	105±14	90±14	95±13	85±12
SBP (mmHg)	129±29	130±31	113±28	126±30	112±30	121±28	112±27	120±29

Table 5 Continued

DBP (mmHg)		68±18	67±19	57±20	66±19	56±21	64±20	59±20	62±19
MAP (mmHg)		88±19	88±21	75±21	86±21	75±22	84±21	77±21	81±20
Temperature (^{o}C)		36.8±2.0	37.2±1.2	36.5±1.3	36.8±1.3	36.4±1.8	36.8±1.4	36.7±1.5	37.2±1.4
Proportion of patients with Temperature < 36^{o}C		0.07	0.06	0.21	0.11	0.26	0.15	0.17	0.07
DRG (n/%)	Medical	236/57.2	287/54.7	84/40.4	353/53.7	27/37.0	385/50.5	207/49.6	99/52.4
	Surgical	155/37.5	203/38.7	113/54.3	267/40.6	43/58.9	325/42.7	188/45.1	75/39.7
	Missing	22/5.3	35/6.6	11/5.2	37/5.6	3/4.1	52/6.8	22/5.3	15/7.9
Time to sepsis (minutes)*		272±306	292±354	245±308	257±294	190±205	227±287	214±266	271±343
Lactate (n=2117)		NA	NA	5.7±5.11	2.5±2.8	7.3±5.9	3.1±2.8	4.1±3.9	NA
Base deficit (mEq/L)		-0.7±5.4	0.8±5.5	7.9±7.6	1.6±6.7	10.6±8.0	3.1±6.2	5.8±6.8	2.0±5.4
pH		7.41±0.08	7.39±0.08	7.28±0.15	7.36±0.11	7.26±0.15	7.35±0.11	7.32±0.13	7.38±0.09
$PaCO_2$ (mmHg)		43.2±15.6	40.4±13.1	38.0±12.9	42.6±14.3	34.1±11.2	40.5±13.5	38.2±11.0	38.5±9.7
PaO_2 (mmHg)		121±77	132±78	155±97	135±87	156±86	144±93	151±95	147±81
SaO_2 (%)		97±4	97±5	94±14	95±8	94±13	96±8	96±7	97±6
Hemoglobin (mg/dL)		11.1±2.2	11.2±2.2	10.9±2.7	11.1±2.2	11.0±3.0	10.8±2.3	10.4±2.4	10.8±2.5
White blood cell count ($1x10^9$/L)		12.7±6.6	13.2±7.2	13.8±9.9	14.9±11.7	18.4±18.21	13.5±8.3	14.6±9.0	13.7±8.2
Proportion of patients with WBC < $4.5x10^9$/L		0.06	0.04	0.13	0.05	0.18	0.10	0.11	0.10
Platelet count ($1x10^9$/L)		229±122	232±127	168±110	215±117	174±130	200±123	184±114	202±122

Table 5 Continued

Glucose (mg/dL)	161±69	159±64	147±70	155±65	145±107	164±73	154±71	180±77
Creatinine (mg/dL)	1.2±1.0	1.0±0.81	1.1±0.72	1.2±1.1	1.1±0.9	1.2±0.8	1.1±0.7	1.0±0.4
Process of care								
Vasopressor use** (n/%)	91/22.0	109/20.8	157/75.5	282/42.9	58/79.5	341/44.8	284/68.1	72/38.1
Mechanical ventilation during first 24 hours (n/%)	177/42.9	296/56.4	184/88.5	474/72.2	70/95.9	571/74.9	355/85.1	121/64.0
RRT ** (n/%)	3/0.7	0/0	9/4.3	8/1.2	3/4.1	8/1.1	9/2.2	0/0
FiO_2	0.41±0.35	0.46±0.28	0.7±0.3	0.6±0.3	0.64±0.33	0.61±0.3	0.63±0.3	0.48±0.31
Fluids administered within first 24 h (L)	1.1±0.61	3.1±0.62	10.1±1.0	1.9±0.82	13.9±1.22	4.32±0.71	7.0±0.83	5.8±0.94
Outcome data								
ICULOS (days)	9.06±15.4	8.9±10.1	13.1±14.4	11.2±16.4	12.2±9.4	12.2±15.7	13.3±15.7	10.2±13.9
HosLOS (days)	19.8±28.1	20.2±18.4	21.4±21.9	22.1±29.8	21.4±18.4	23.7±26.2	24.5±28.0	19.9±20.7
Mortality 90 days (n/%)	135/32.7	127/24.2	88/42.3	250/38.1	36/49.3	262/34.4	171/41.0	73/38.6
Mortality 365 days (n/%)	192/46.5	182/34.7	101/48.6	315/47.9	41/56.2	341/44.8	220/52.8	83/43.9

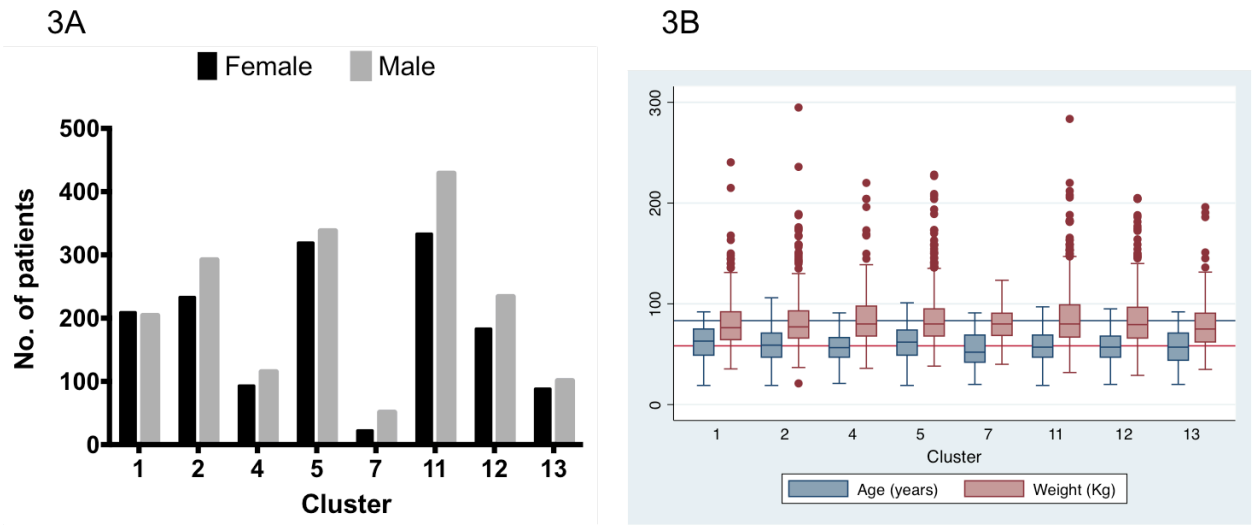
RRT=Renal replacement therapy; *=Time from admission to establishment of diagnosis of "suspected sepsis"; **=at admission; FiO_2 = Inspired fraction of Oxygen

Demographic data. In this section the population will be described in terms of their age, weight, and gender distribution per cluster. Overall, the majority of patients were males. In the per

cluster analysis, only cluster No. 1 nominally had slightly more females than males (50.6 vs. 49.4%). However, this difference was not statistically significant. The clusters were the difference between females and males was most notable, were clusters 2 (44.4 vs. 55.6%), 7 (30.1 vs. 69.9%), 11 (43.7 vs. 56.3%), and 12 (43.9 vs. 56.1%) (Figure 3A).

With regards to age, clusters 1 and 5 had the oldest population with mean age in years of 60.9±17.1 and 60.9±16.7, respectively. The population age in clusters 4, 7, 11 and 12 had significantly different from those in clusters 1 and 5, with 56.2±14.7, 53.4±17.4, 57.1±16.2 and 55.9±15.9, respectively.

Although statistically, there was a significant difference between clusters in terms of weight ($p=0.03$), it is unlikely that is a relevant one clinically. The group with the heaviest population was cluster 4 with mean weight of 85.6±29.5 kg. The cluster with the lightest population was cluster 13, with 79.4±26 Kg. Figure 3B shows age and weight per cluster.



3A. Gender; 3B. Age and weight. Red and blue continuous lines represent mean age and weight for the entire cohort respectively.

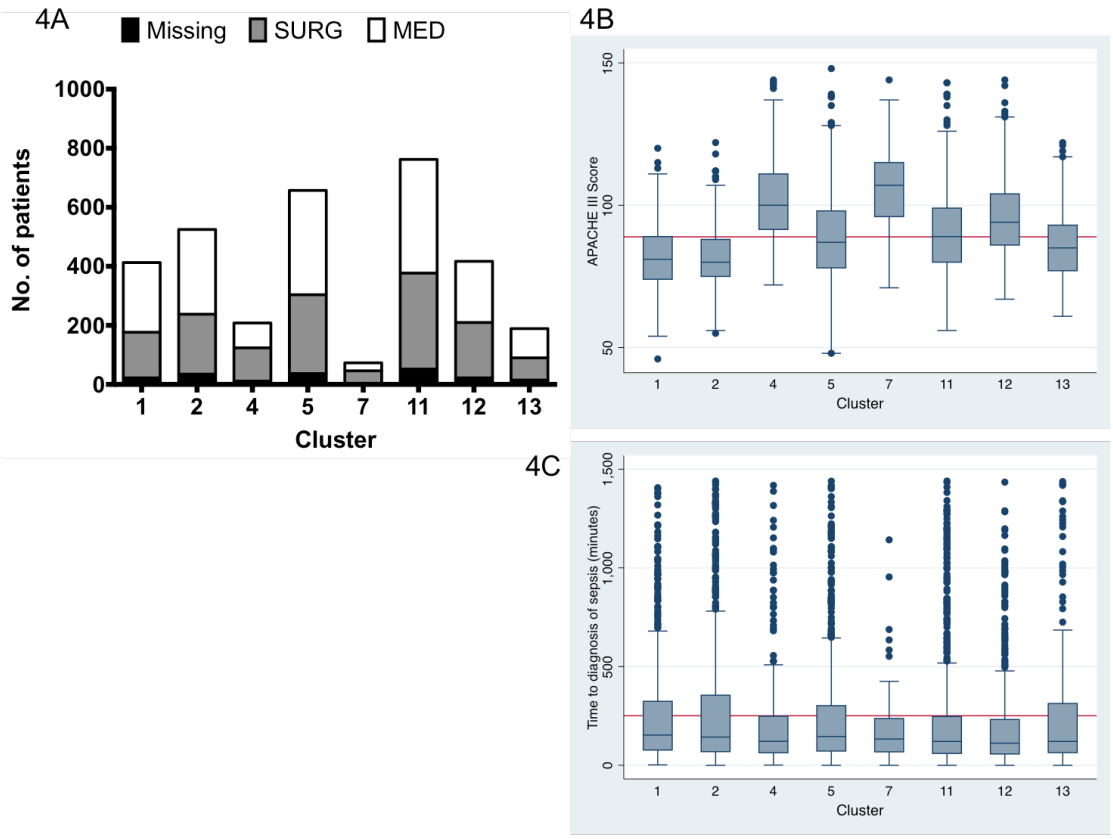
Figure 3. Gender, age and weight per cluster.

Clinical and physiologic data: There was a significant difference in the distribution of patients by diagnosis related group codes (DRG codes) per cluster. The DRG code is a system to classify hospital cases into specific groups taking into account International Classification of Diseases (ICD) diagnoses, procedures, age, sex, discharge status and presence of complications and comorbidities[14]. These codes are categorized broadly into "service line categories", namely Medical, Surgical or Maternity care. In the studied population, no patients were allocated to the "Maternity care" category given that patients with DRG codes related to this group are rarely admitted to the hospitals were the HiDenIC-8 database was collected from. As the categories denote, overall medical patients are individuals who are suffering from a disease process, and have procedures and complications not related to surgical interventions. In opposition, surgical

patients are by definition, patients that have required a surgical intervention. This distinction is important, as these patients are known to have distinct trajectories in terms of acuity, severity and outcome. The selected cohort of patients in the present study showed allocation to one of three groups: Medical, Surgical, or Missing data. The number of patients allocated to the "Missing data" group was small (n=197/3244, 6.1% of the total population). A total of 1678 (51.7%) patients were allocated to Medical, and 1369 (42.2%) to the Surgical categories. With the exception of clusters 4 and 7, all clusters had more medical than surgical patients as Figure 4A shows.

Figure 4B, shows the severity of the disease process as quantified by the Acute Physiology, Age, Chronic Health Evaluation or APACHE III score[15]. The APACHE III is a five point scoring system (range between 0 and 299), which has been validated and accurately predicts the risk of death (area under receiver operating characteristic curve of 0.9) when measured in the first 24 hours after ICU admission. Overall, APACHE III scores were different between clusters ($p = 0.0001$). Clusters 4 and 7 were distinctly higher than the rest, being the only groups with scores above 100 (cluster 4, 102±15.2; cluster 7, 105±14.3).

The time in minutes between reaching the criteria for "suspected sepsis" and ICU admission was identified as "Time to Sepsis". Although, overall there was a significant difference between clusters, the actual differences were probably not clinically significant. The average Time to sepsis for most of the clusters was between 214 and 292 minutes, with the exception of cluster 7, which was the shortest with 190 minutes. There was however, important variation as it ranged from 0 to 1440 minutes (24 hours). (Figure 4C)



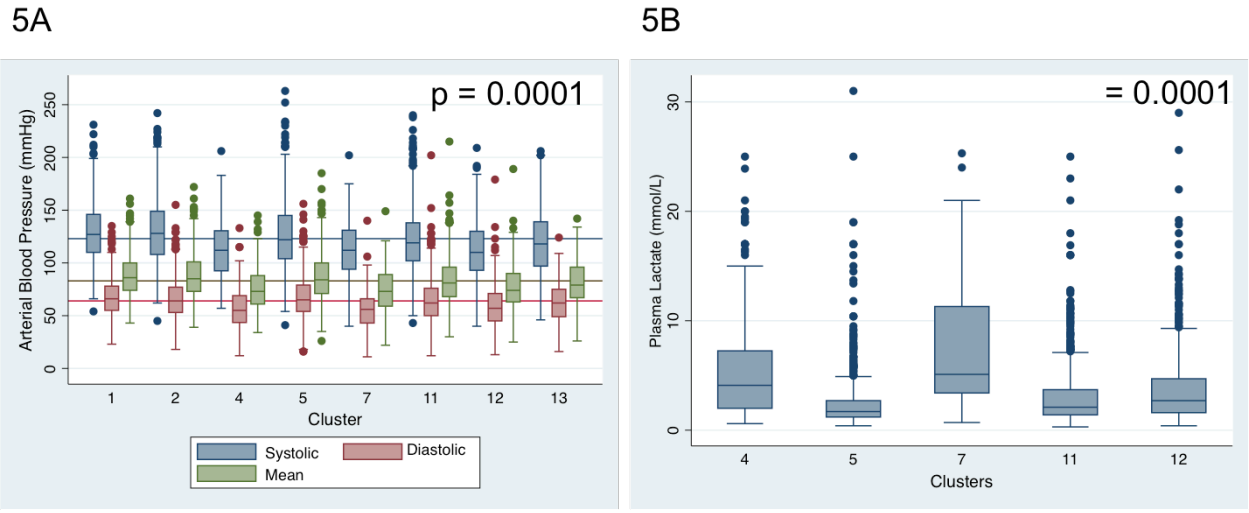
4A. Diagnostic Related group classification per cluster. MED = Medical; SURG=Surgical. 4B. APACHE III scores per cluster. Red continuous lines represent mean APACHE III score. 4C. Time to diagnosis of sepsis per cluster. Red continuous lines represent mean Time to diagnosis of Sepsis, respectively.

Figure 4. Diagnostic Related group, APACHE III score and Time to diagnosis of sepsis per cluster.

The remainder of the physiologic data will be presented by organ/system.

Cardiovascular system. The systolic, diastolic and mean arterial pressures are shown in Figure 5A. The lowest mean arterial pressures (MAP) were found in cluster 7 (74.9±22.7 mmHg), 4 (75.9±21.4 mmHg) and 12 (76.9±20.5 mmHg), whereas the rest of the clusters had MAP above 80. Admission lactate was not available for clusters 1, 2, and 13. Of the patients from clusters that did have an ICU admission lactate available, most of them were abnormally high (i.e. > 2

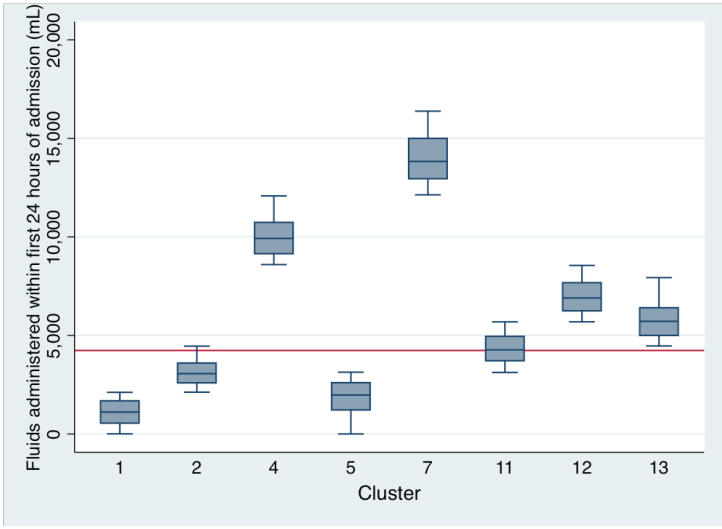
mmol/L). Cluster 7 had the highest plasma level (7.3±5.9 mmol/L), followed by cluster 4 (5.7±5.1 mmol/L), and the lowest was seen in cluster 5 (2.5±2.8 mmol/L). Figure 5B shows mean±SD lactate concentrations in blood per cluster. In addition, clusters 4, 7 had the highest intravenous fluid requirements of the entire cohort (Figure 5C). These two clusters, as well as cluster 12 and 13, were all above the cohort average fluid use in the first 24 hours after ICU admission of 4.2L. Cluster 1 had the lowest fluid use.



5A. Systolic, diastolic and mean arterial pressure at admission per cluster. Blue, Green and Red continuous lines represent average systolic, mean and diastolic arterial pressure at admission for the entire cohort. 5B. Plasma lactate per cluster. P values reported are from either ANOVA or Kruskal-Wallis test depending on normality of distribution.

(**Figure 5** continued below)

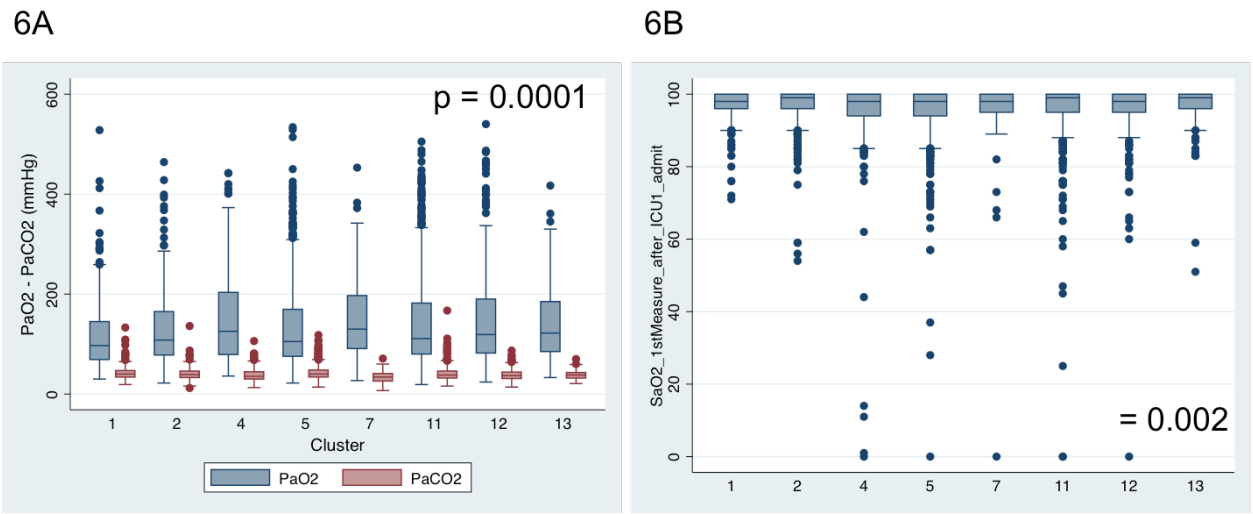
5C



5C. Administration of intravenous fluids per cluster in the first 24 hours after ICU admission in mL. Red continuous line represents mean fluid administration for the entire cohort in the first 24 hours.

Figure 5. Cardiovascular system variables at admission per cluster.

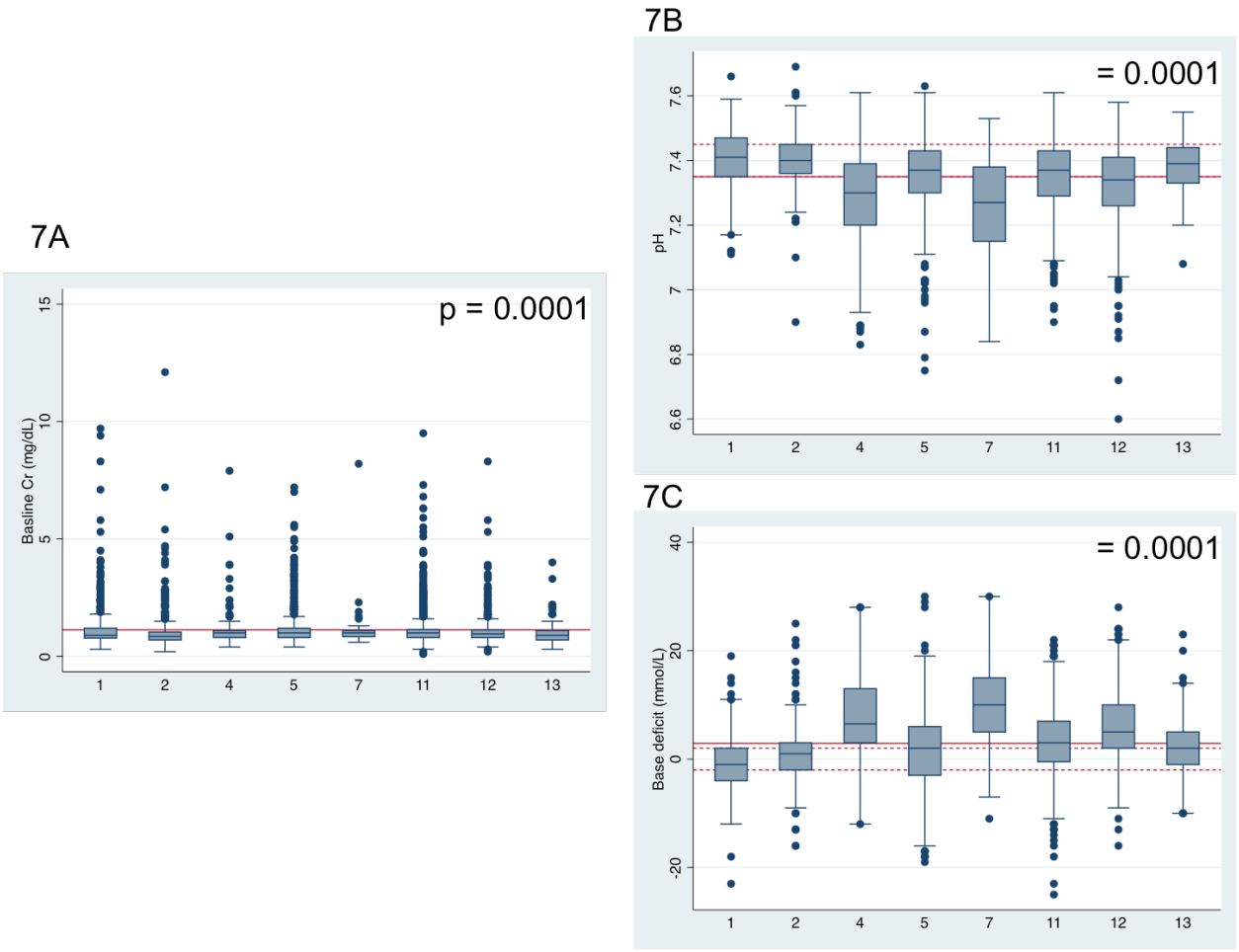
Respiratory system. From the respiratory standpoint, the clusters appeared to have similar arterial blood gases in terms of arterial partial pressure of O2 (PaO2), CO2 (PaCO2) and saturation (SaO2). Cluster 1 had the lowest PaO2, and the highest quantitatively were clusters 4, 7 and 12. In terms of mean SaO2, interestingly the lowest was found in clusters 4 and 7 (94.0±13.9, 94.9±13%, respectively). (Figure 6A and B)



6A. Arterial O2 and CO2 partial pressure per cluster. 6B. Arterial saturation of O2 per cluster. P values reported are from either ANOVA or Kruskal-Wallis test depending on normality of distribution.

Figure 6. Respiratory system variables at admission per cluster.

Renal system. We used the last recorded Creatinine in the previous year as a measure of baseline value. Cluster 5 had the highest baseline Creatinine, followed by cluster 1 (1.24±1.13 and 1.21±1.03 mg/dL). The rest of the clusters showed mean Creatinine values below 1.2 mg/dL (Figure 7A). In terms of acid base balance, clusters 4 and 7 had the lowest pH and highest base deficits (BD) (7.26±0.15 vs. 7.28±0.14, and 10.5±7.9 vs. 7.9±7.6 mmol/L, respectively), suggesting a metabolic acidosis type of derangement upon admission to the ICU. Clusters 4, 7, 11 and 12 had BD above the normal upper limit of 2 mmol/L. The clusters 1, 2, 5, and 13 had pH and BD within normal limits (Figure 7B and C).

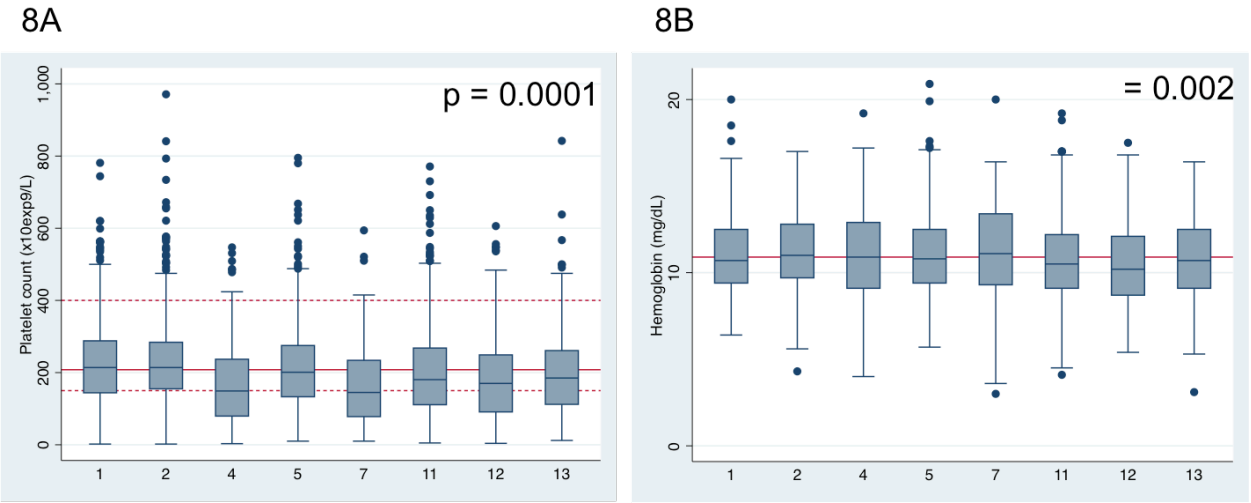


7A. Baseline creatinine per cluster. 7B. pH per cluster. 7C. Base deficit per cluster. Red discontinuous lines represent lower and upper limits of normality for pH and Base deficit. Red continuous lines represent mean creatinine, pH and base deficit for the entire cohort.

Figure 7. Renal system variables at admission per cluster.

Hematologic system. There were no clinically significant differences in the level of hemoglobin at admission to the ICU between clusters (Figure 8A). No cluster showed mean hemoglobin levels below 10 mg/dL. In terms of platelets, all clusters had relatively normal mean counts upon admission to the ICU. No cluster was found to have a platelet count below the normal range (150

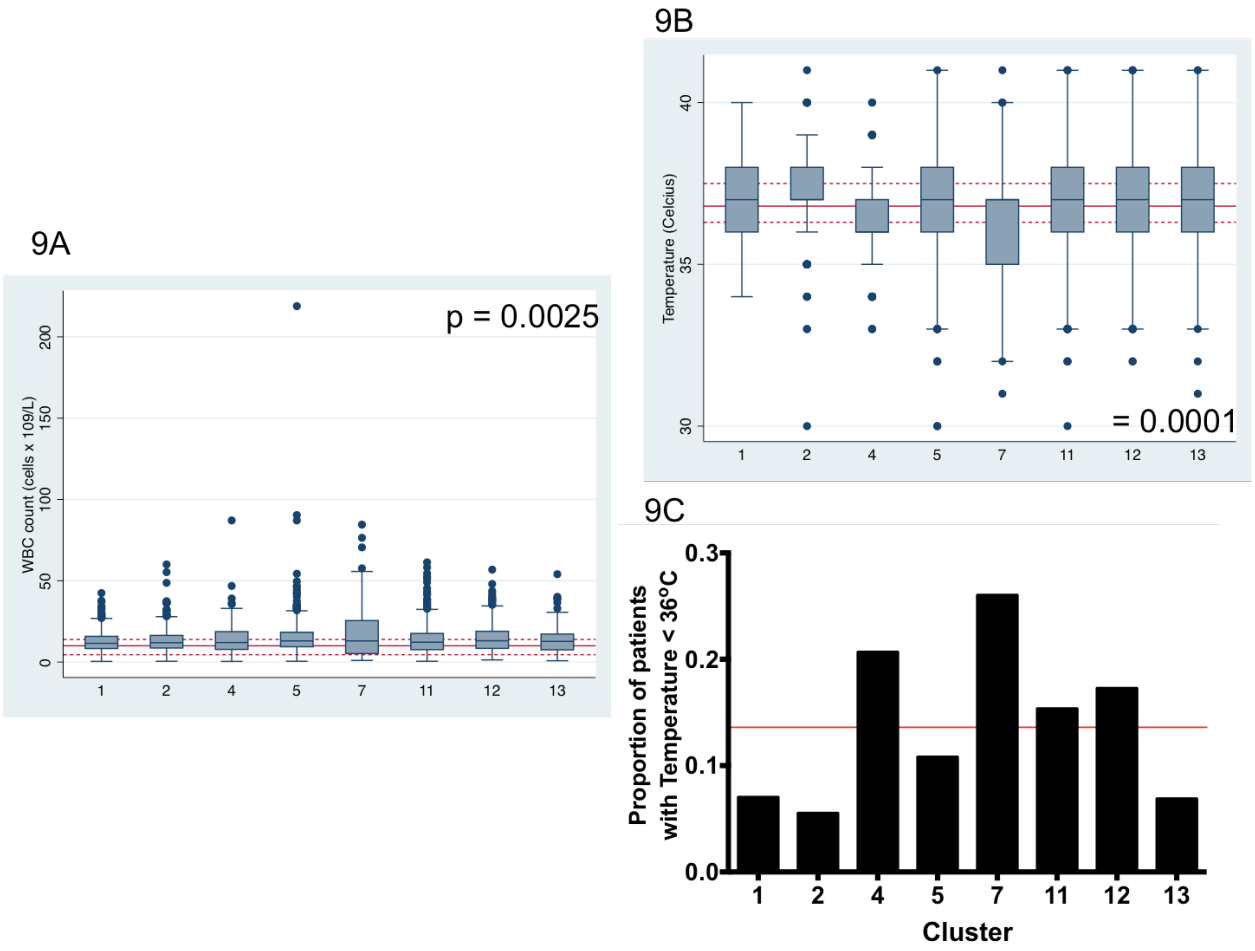
– 400 x 10^9/L) as shown in Figure 8B. Average platelet count however, was below 200 x 10^9/L in clusters 4, 7 and 12.



8A. Platelet count per cluster. 8B. Hemoglobin concentration in blood per cluster. Red continuous lines represent mean platelet and hemoglobin values for the entire cohort. Red discontinuous lines represent range of normality.

Figure 8. Hematologic system variables at admission per cluster.

Immune and metabolic response. The mean white blood cell count (WBC) on admission for the entire population was 13.9±9.3 x 10^9/L. Cluster 7 had the highest WBC, followed by cluster 5 and 12 (18.3±18.2, 14.9±11.8, 14.6±8.9 x 10^9/L, respectively). In terms of temperature at admission, the highest mean temperature was found in cluster 13 (37.2±1.4^{o}C), and the lowest in cluster 7 (36.4±1.8^{o}C). Figures 9A and B show WBC and temperature at admission per cluster. Cluster 7 had the highest proportion of patients with temperature below 36^{o}C, followed by clusters 4 and 12 (0.26, 0.21, and 0.17, respectively. Figure 9C).



9A. White blood cell count per cluster. 9B. Body temperature at admission to the ICU per cluster. 9C. Proportion of patients with body temperature below 36°C at admission per cluster. Red continuous line represents mean values for the entire cohort. Red discontinuous lines represent range of normality.

Figure 9. Immunologic and metabolic response variables at admission per cluster.

A Multinomial logistic regression was used to assess which variables better defined each cluster (See Appendix B1). Vasopressor use, the need for mechanical ventilation within the first 24 hours of admission to the ICU and admission temperature were the most important predictors of cluster membership according to the multinomial logistic regression coefficient yield. All clusters, except for cluster 1 had platelet count and APACHE III score as a predictor of

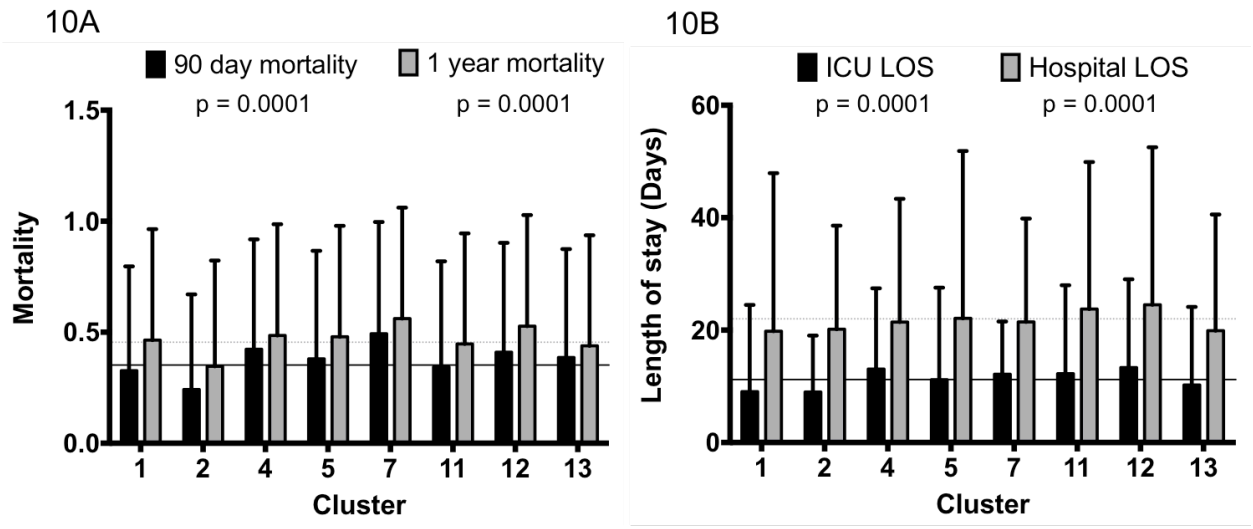
membership. Baseline creatinine was an important predictor of membership only in clusters 1 and 5. Age, was an important predictor of membership in clusters 4, 7, 11 and 12. Finally, WBC was a predictor of membership only in clusters 5, 7 and 12.

3.2 ASSOCIATION OF CLUSTERS WITH OUTCOME MEASURES

3.2.1 Association with mortality and length of stay

Both mortality at 90 and 365 days were different between clusters as demonstrated in Figure 10A. The highest 90-day mortality was found in cluster 7, followed by 4 and 12 (0.49, 0.42 and 0.41, respectively). These same three clusters had the highest 365-day mortality, with cluster 7 being the highest (0.56). However, 365-day mortality was higher in cluster 12 than in cluster 4 despite (0.52, 0.48, respectively) cluster 4 having a higher 90-day mortality.

Intensive care unit (ICULOS) and hospital length of stay (HosLOS) per cluster were also studied. Mean ICULOS and HosLOS were 11.2±14.8 and 22.0±25.7 days, respectively. Four clusters were above the mean ICU LOS for the entire cohort, with cluster 12 having the longest mean stay, followed by 4, 11 and 7 (13.3±15.7, 13.1±14.4, 12.3±15.7, 12.1±9.4, respectively). Only two clusters, 12 and 11, had HosLOS beyond the cohort mean (24.5±27.9 and 23.7±26.2, respectively).(Figure 10B)



10A. Ninety day and 1 year mortality per cluster. 10B. ICU and hospital length of stay per cluster. Continuous black line represents mean 90-day and ICULOS for the entire population. Gray discontinuous line represents mean 1-year and Hospital LOS for the entire cohort.

Figure 10. Mortality and hospital and ICU length of stay per cluster.

3.2.2 Association with organ dysfunction and patterns of organ/system compromise

The association of each cluster with organ dysfunction was also explored. Organ/system dysfunction was assessed as shown in table 2 at two different time points: 72 hours and 30 days. Based on the pre-specified decision rules (Appendix A2), the number of organs, the pattern of organ compromise and the trajectory of each cluster in terms of clinical course were determined. The number of organs compromised, and the pattern of involvement per cluster for the 72-hour and 30 day time points are shown in Table 6 and 7, respectively. The most frequently compromised organs/systems were the Cardiovascular (6 clusters), Respiratory (5 clusters), and Hepatobiliary systems (5 clusters). The renal and Hematologic systems were compromised in 3 clusters. Only cluster 2 had no organ compromise at 72 hours. Clusters 1 and 13 had 1 organ

compromised; cluster 5 had 2 organs; cluster 11, 3 organs; and finally, clusters 4, 7, and 12 had 5 organs compromised. Results will be presented below according to each organ/system assessed.

Table 6. Organ compromise at 72 hours per cluster

	Clusters													
Organ/system	**1**		**2**		**4**		**5**		**7**		**11**		**12**		**13**	
Cardiovascular	Yes		No		Yes[#]		Yes		Yes[#]		Yes		Yes[#]		No	
Lactate (mmol/L)*	3.1	+	2.4	-	4.6	+	2.0	-	5.5	+	2.3	-	3.1	+	1.9	-
Vasopressor use	-		-		+		+		+		+		+		-	
Pulmonary	No		No		Yes		Yes		Yes		Yes		Yes		No	
MV	-		-		+		+		+		+		+		-	
PaO2/FiO2	222	-	217	-	203	-	206	-	208	-	218	-	228	-	216	-
Renal	No		No		Yes		No		Yes[#]		No		Yes		No	
Δ in Cr (mg/dL)*	0.4/R	-	0.2/0	-	0.7/R	-	0.6/R	-	1/I	+	0.4/ R	-	0.5/R	-	0.3/ R	-
RRT	-		-		+		-		+		-		+		-	
Hematologic	No		No		Yes[#]		No		Yes[#]		No		Yes[#]		No	
Δ Platelet count* ($x10^3$/L)	-17	-	-30	-	-60	+	-38	-	-99	+	-43	-	-58	+	-30	-
Hepatobiliary	No		No		Yes[#]		No		Yes[#]		Yes		Yes[#]		Yes[#]	
Total Bilirrubin (median, mg/dL)*	0.9	-	1.3	-	4.3	+	1.6	-	4.8	+	2.4	+	4.2	+	3.8	+
INR	1.4	-	1.3	-	1.6	+	1.4	-	1.7	+	1.4	-	1.6	+	1.5	+

Table 6 Continued

Total of organs	1	0	5	2	5	3	5	1
Compromise with criteria in "severe" range			CV, R, Hm		CV,R,Hm, Hp		CV, Hm, Hp	Hp

*=Value of each variable | assignment (if organ dysfunction criteria reached then "+"; if not, then "–"). # = Reached organ dysfunction criteria with values denoting severe compromise. Shaded gray area represents organ dysfunction criteria. Yes/No denomination = "Yes", was assigned if organ dysfunction criteria was reached for the system in question for each cluster. "No", was assigned if it did not.

Table 7 shows the breakdown for each organ/system per cluster at the 30-day time point.

Table 7. Organ compromise at 30 days per cluster

	Clusters															
Organ/system	**1**		**2**		**4**		**5**		**7**		**11**		**12**		**13**	
Cardiovascular	No		No		No		No		Yes		No		Yes		No	
Vasopressor use	-		-		-		-		+		+		+		-	
Pulmonary	No		No		Yes		Yes		Yes		Yes		Yes		Yes	
MV	-		-		+		+		+		+		+		+	
PaO_2/FiO_2*	284	-	257	-	177	+	179	+	196	+	239	-	255	-	157	+
Renal	No		No		Yes		Yes		Yes		No		Yes		Yes	
Δ in Cr (mg/dL / RIFLE category)*	0.3/R	+	0.04/0	-	0.35/R	+	0.35/R	+	0.42/I	+	0.23/R	-	0.25/R	-	0.48/R	+

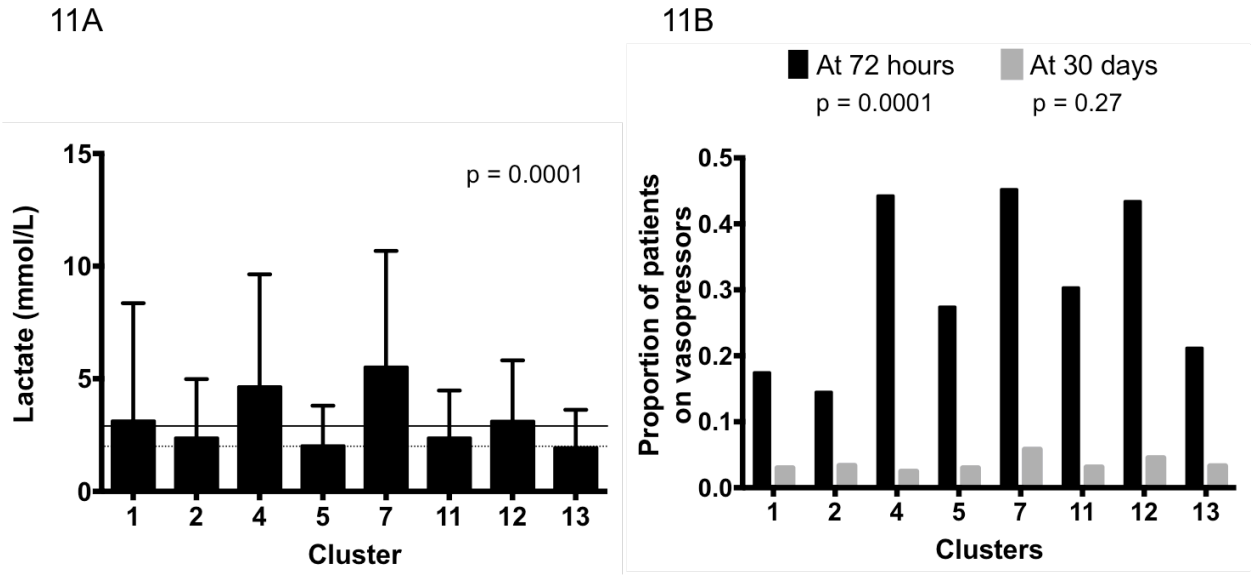
Table 7 Continued

RRT	-		-		+		+		+		-		+		+	
Hematologic	No		No		No		No		No		No		No		No	
Platelet count ($x10^9$/L) *	225	-	206	-	228	-	236	-	248	-	218	-	178	-	165	-
Hepatobiliary	No		No		No		No		Yes		No		Yes		Yes	
Total Bilirrubin (median, mg/dL) *	1.0	-	1.9	-	1.9	-	1.6	-	14.4	+	1.4	-	3.9	+	12	+
Total of organs	0		0		2		2		4		1		4		3	

*=Value of each variable | assignment (if organ dysfunction criteria reached then "+"; if not, then "–"). RIFLE category: 0=Normal; R=Risk, I=Injury. Shaded gray area represents organ dysfunction criteria. Yes/No denomination = "Yes", was assigned if organ dysfunction criteria was reached for the system in question for each cluster. "No", was assigned if it did not.

Cardiovascular system: Lactate was selected as global measure of organ perfusion and thus of cardiovascular sufficiency. Average lactate levels at 72 hours for the entire cohort was 2.9±3.3 mmol/L (normal values < 2 mmol/L). Patients in Cluster 7 had significantly elevated lactate levels after 72 hours of admission to the ICU, and registered as the highest levels of the entire cohort, followed by Cluster 4 (5.5±5.2 and 4.6±5.0 mmol/L, respectively; See Figure 11A). The lowest 72 hour mean lactate level was found in cluster 13, which was normal, followed closely by clusters 5 and 2 (1.9±1.7, 2.0±1.8, 2.4±2.6 mmol/L respectively).

The need for vasopressors was explored also as a measure of cardiovascular compromise and was quantified as either being on or off any vasopressor or inotrope at 72 hours and at 30 days after ICU admission. Clusters 7, 4 and 12 had the highest proportion of patients requiring vasopressors or inotropes in the first 72 hours after ICU admission as shown in Figure 11B (0.45, 0.44 and 0.43). The lowest requirement of cardiovascular support was seen in clusters 2 and 1 (0.14 and 0.17). At 30 days the proportion of patients on vasopressor or inotropic support decreased 5-10 fold in every cluster as expected (Figure 11B). The pattern persisted though, with clusters 7 and 12 showing the highest requirement of pharmacologic cardiovascular support (0.06 and 0.05, respectively). However, cluster 4 showed a decrease in the proportion of patients on vasopressors or inotropes at this time point.



11A. Blood lactate at 72 hours per cluster. Black continuous line represents mean lactate concentration for the entire population. Black discontinuous line represents upper limit of normality. 11B. Proportion of patients requiring vasopressor use at 72 hours and 30 days after admission to the ICU. P values for proportions derived from logistic regression.

Figure 11. Cardiovascular system variables at 72 hours and 30 days per cluster.

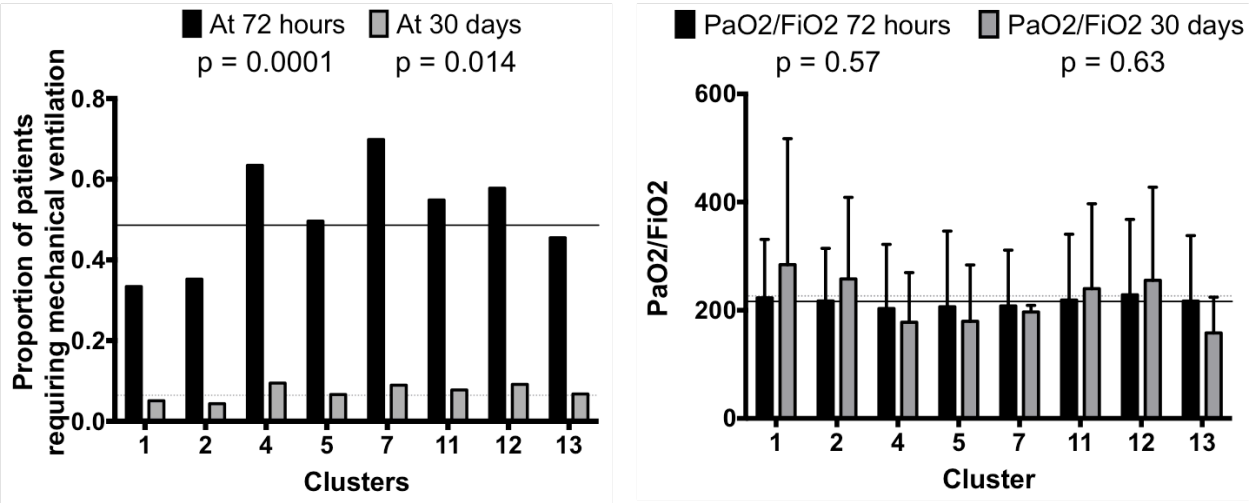
Respiratory system: The proportion of patients requiring mechanical ventilation in the entire cohort at 72 hours was 48.6%. Clusters 7, 4 and 12 had the highest proportion of patients requiring mechanical ventilation at 72 hours (0.69, 0.63 and 0.58, respectively). As expected, only 6.4% of the entire population required mechanical ventilator support at 30 days. However, the pattern persisted, with clusters 7, 4 and 12 having the highest proportion of ventilator dependent patients (see Figure 12A).

The ratio between the partial pressure of O2 and the delivered fraction of O2 (PaO2/FiO2 ratio) was also calculated to assess the extent of pulmonary compromise. Although all these patients did not have acute respiratory distress syndrome, we used the Berlin definition[16] to gauge and classify the extent of the oxygenation compromise according to the PaO2/FiO2 ratio like this:

- Mild: PaO2/FiO2 ratio between 200-300
- Moderate: PaO2/FiO2 ratio between 100-200
- Severe: PaO2/FiO2 ratio below 100

At 72 hours, the mean PaO2/FiO2 ratio for the entire cohort was 216±125 (n=580 observations), and at 30 days 226±146 (n=95 observations). Cluster 12, followed by 11 and 1 had the highest PaO2/FiO2 ratios at 72 hours, whereas 4,5 and 7 had the lowest. Similarly, clusters 1, 2, 11 and 12, had the highest PaO2/FiO2 ratios at 30 days, while 4, 5, 7 and 13 had the lowest as shown in Figure 12B.

12A 12B

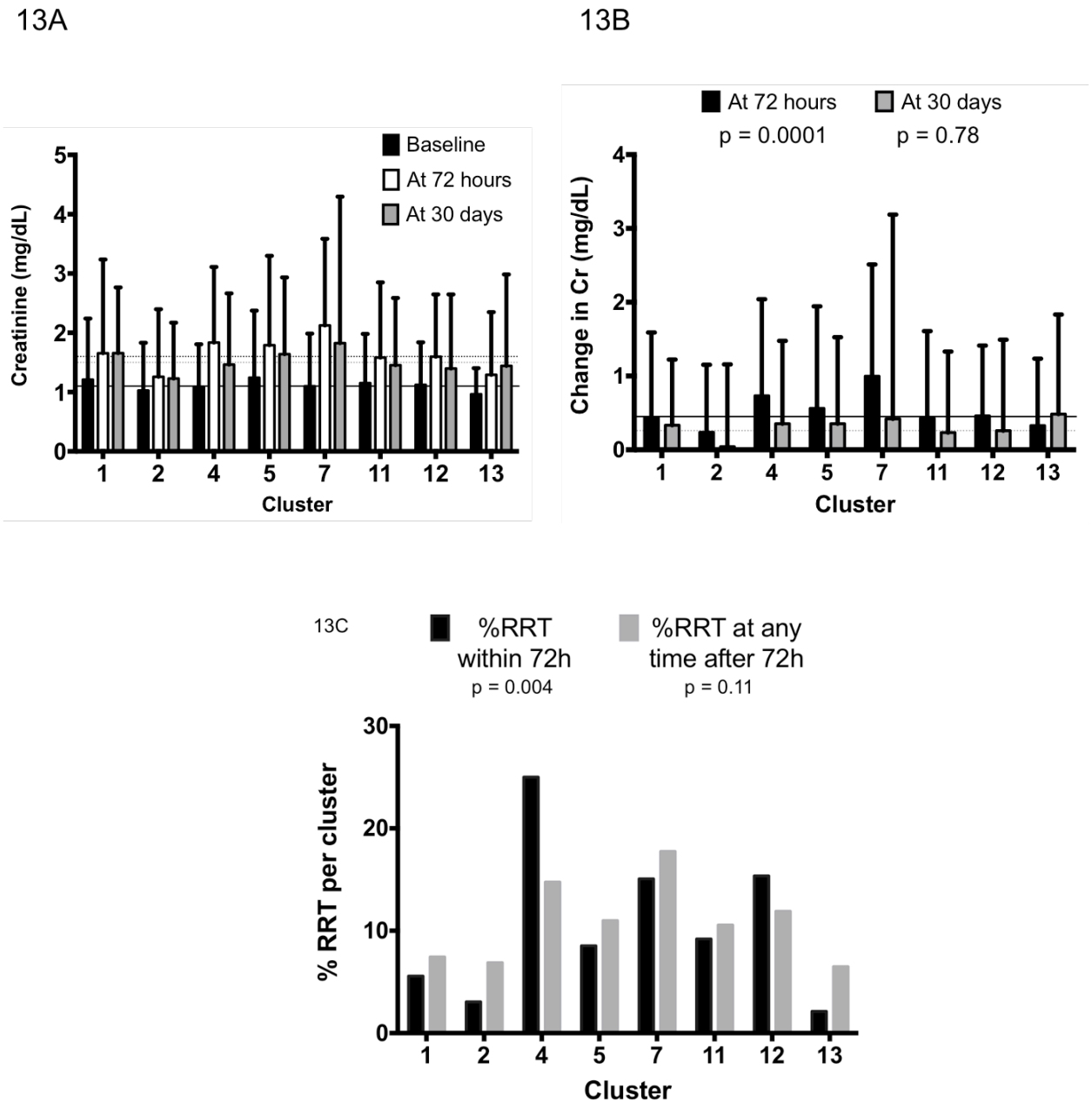


12A. Proportion of patients requiring mechanical ventilator support at 72 hours and 30 days after ICU admission. 12B. PaO2/FiO2 ratio at 72 hours and 30 days after ICU admission. Black continuous line represents mean values for the entire cohort at 72 hours. Gray discontinuous line represents mean values for the entire cohort at 30 days. P values for proportions derived from logistic regression.

Figure 12. Respiratory system variables at 72 hours and 30 days per cluster.

Renal system: Renal function and compromise was assessed by measuring creatinine at baseline, 72 hours and 30 days, by assessing the change in Creatinine from baseline to the pre-specified timepoints, and by assessing the need of renal replacement therapy (RRT) at 72 hours and 30 days. Figure 13A shows the levels of Creatinine at baseline, and 72 hours and 30 days after admission to the ICU. Mean baseline, 72 hour and 30 day creatinine levels for the entire cohort were 1.10±0.89, 1.59±1.33 and 1.48±1.22 mg/dL, respectively. Baseline creatinine values were significantly different between clusters (p=0.0001), with clusters 5 and 1 having the highest variability. However, mean values were rather similar, and differences were not clinically significant. More important to evaluate the impact of sepsis on renal function was the change in

creatinine, shown in Figure 13B. Clusters 7, 4 and 5 had the largest creatinine increase at 72 hours. The RIFLE classification of acute kidney injury[17] has been extensively validated, and is pertinent as it is associated with adverse renal outcomes such as dialysis, non-recovery of renal function and death. These instrument classifies the renal injury in progressive categories as R (Risk), I (Injury), F (Failure), L (Loss) and E (End stage kidney disease). It is based on serum creatinine and urine output. In this study, only the creatinine criterion was explored. The RIFLE system classifies an acute rise in creatinine of 0.3 mg/dL as R, or being "at risk" for AKI. An increment in creatinine that doubles baseline values is considered as I, or Injury. At 72 hours, all clusters except cluster 2 had criteria for RIFLE R classification. However, cluster 7 showed almost doubling of creatinine levels at this time point, suggesting these patients were more severely compromised from the renal stand point (increase in creatinine 1.93 times the mean baseline for the cluster). In addition, cluster 4's mean baseline creatinine increased 1.7 times, suggesting as well higher level of renal compromise. At 30 days, cluster 7 had the highest change from baseline (1.7 times), followed by cluster 13 (1.5 times). All other clusters showed increments in creatinine below 1.5 times the baseline suggesting renal recovery.



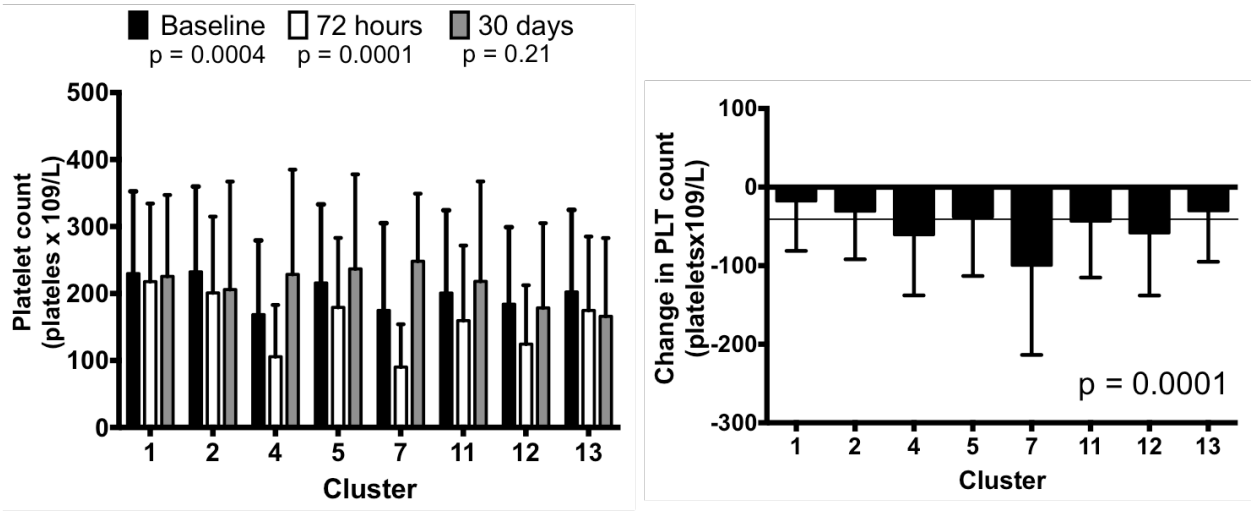
13A. Serum Creatinine baseline (black), at 72 hours (white) and at 30 days (gray) after ICU admission. Continuous black lines represent mean baseline creatinine value for the entire population. Discontinuous black and gray lines represent mean creatinine values for 72 hour and 30 day timepoint for the entire cohort, respectively. 13B. Change in creatinine calculated as creatinine level at 72 hours (black bars) or 30 days (gray bars) after ICU admission minus baseline creatinine. Black continuous bar represents mean creatinine change at 72 hours for the entire cohort. Gray discontinuous line represents mean creatinine change at 30 days for the entire cohort. 13C. Percentage of patients per cluster requiring RRT within the first 72 hours of admission to the ICU (black bars), and between 72 hours and 30 days (gray bars).

Figure 13. Renal system variables at 72 hours and 30 days per cluster.

The need for RRT was also explored as a measure of renal compromise. Figure 13C shows the proportion of patients requiring RRT at 72 hours and then at any time after 72 hours. Clusters 4, 12 and 7 had the highest percentage of patients requiring RRT at 72 hours (25, 15.3, and 15.1%, respectively). As expected, clusters 4, 7 and 12 had the highest requirement of RRT beyond 72 hours. Of these clusters, the only cluster that did not show improvement (i.e. a decrease in % of patients on RRT beyond 72 hours) was cluster 7. Importantly, clusters 2 and 13 had a doubling of the need for RRT when the 72 hours time point is compared to beyond 72 hours.

Hematologic system. Platelet count was the mainstay of the assessment. Admission (baseline), 72 hour and 30 day platelet counts for the entire cohort were 207±122, 167±110 and 214±141, respectively. Figure 14A shows baseline, 72 hour and 30 day platelet counts per cluster. Severity of compromise was related to platelet count drop after admission to the ICU, and it was quantified as the change in platelets from baseline to 72 hours, as shown in Figure 14B. In general, all clusters showed an average decline in platelet count of -41±73x10^9/L. Cluster 7, 4 and 12 displayed the largest decline in platelet count (-99, -60 and -58 x10^9/L). Only cluster 7 reached a mean platelet count below 100 x 10^9/L.

14A 14B

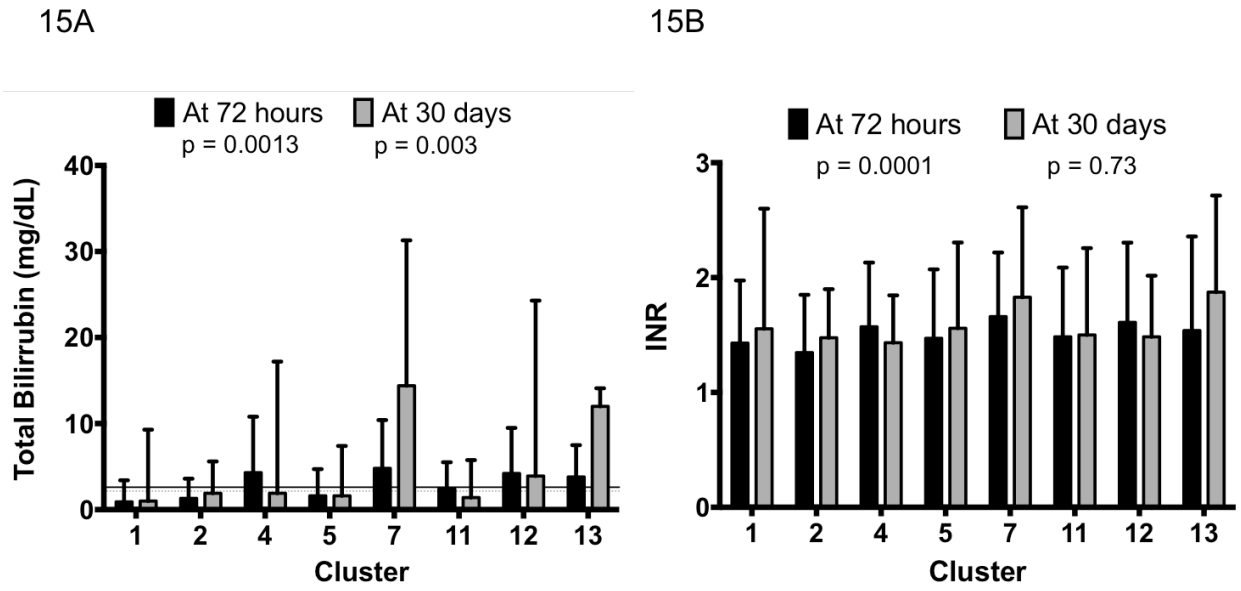


14A. Platelet count at admission (baseline), 72 hours and 30 days after admission to the ICU. 14B. Change in platelet count calculated as Platelet count at 72 hours minus baseline platelet count. Black continuous line represents mean change in platelet count for the entire cohort.

Figure 14. Hematologic system variables at 72 hours and 30 days per cluster.

Hepatobiliary system. Total bilirubin levels and the International Normalized Ratio or INR were used to assess hepatic compromise. Total bilirubin at 72 hours and 30 days was elevated for the entire cohort, suggesting high incidence of hepatic compromise in this population (5.3±7.3 and 7.3±10.4 mg/dL). Of note, for all clusters total bilirubin levels at 30 days were higher than at 72 hours. Clusters 7, 4 and 12 presented the highest total bilirubin levels at 72 hours. In addition to these same clusters, cluster 13 presented the highest total bilirubin levels at 30 days (Figure 15A).

International Normalized Ratios did not differ much between 72 hours and 30 days, with mean values of 1.49±0.6 and 1.52±0.70, respectively. There were significant differences in the by cluster analysis, with cluster 7, 4 and 12 having the highest levels at 72 hours, and 7 and 13 at 30 days (Figure 15B)



15A. Total bilirubin levels at 72 hours and 30 days per cluster. Black continuous line represents mean total bilirubin at 72 hours for the entire cohort. Gray discontinuous line represents mean total bilirubin at 30 days for the entire cohort. 15B. INR at 72 hours and 30 days per cluster.

Figure 15. Hepatobiliary system variables at 72 hours and 30 days per cluster.

When comparing the trajectory of organ compromise from 72 hours to 30 days, the results showed that almost all clusters (with exception of cluster 7 and 12), had resolution of the cardiovascular compromise. Both clusters 7 and 12 however, remained dependent on vasopressors.

From a respiratory stand point, all clusters that showed pulmonary dysfunction at 72 hours, where still ventilator dependent at 30 days, which suggests not only real dysfunction, but inability to recuperate. Cluster 13 had an increase in ventilator use above average at 30 days despite not having it at 72 hours, which may suggest complications during hospital stay affecting the respiratory system, which are not uncommon.

Interestingly, one month after admission, patients in clusters characterized by significant increments in creatinine from baseline, although regressing, where still in average about 0.26 mg/dL from baseline, with clusters 4, 5, 7, and 13 being even above 0.3 mg/dL. The percentage of patients requiring RRT between 72 hours of admission and 30 days was slightly higher than the percentage within the first 72 hours. Importantly, cluster 13 showed a marked rise in RRT need at 30 days, probably alluding to complications of critical illness given that at 72 hours the proportion of patients needing RRT was 3 times less.

All patients with hematological alterations given by platelet count drop recovered by 30 days, suggesting this is a marker of acute disease. No cluster at 30 days had a mean platelet count below 200 x 10^9/L. Finally, only clusters 4 and 11 showed recovery of the hepatobiliary system as measured by total bilirubin levels.

3.2.3 Patterns of organ/system compromise

Based on the pre-specified decision rules (Appendix A2), the number of organs, the pattern of organ compromise and the trajectory of each cluster in terms of clinical course were determined. Table 6 shows the summary of the number of organs compromised, and the pattern of involvement per cluster for the 72-hour time point. The most frequently compromised organs/systems were the Cardiovascular (6 clusters), Respiratory (5 clusters), and Hepatobiliary systems (5 clusters). The renal and Hematologic systems were compromised in 3 clusters. Only cluster 2 had no organ compromise at 72 hours. Clusters 1 and 13 had 1 organ compromised; cluster 5 had 2 organs; cluster 11, 3 organs; and finally, clusters 4, 7, and 12 had 5 organs compromised.

Although clinically multisystem organ failure is defined by compromise of two or more organ/systems[4], patients with two compromised organs had more similarities with those with one organ dysfunction, than patients who had 3 or more. Thus, a comparison between clusters characterized by 1-2 and those with 3 or more organ/system dysfunctions was done. Table 8 shows the summary of all demographic, clinical, process of care and outcome variables compared between clusters with 1-2 organ dysfunctions vs. those with >=3.

Not surprisingly, APACHE III score, ICULOS, HosLOS and mortality at 90 and 365 days are different between these two sets of clusters, with those associated with 3 or more dysfunctional organs having higher scores, stay and mortality, respectively as shown in table 8. In terms of demographic data, clusters with 1-2 organ dysfunctions had more females, more comorbidities and less number of transplants.

Table 8. Demographic, clinical/physiologic, process of care and outcome data per cluster

		Groups by number of compromised organs									
		1 – 2				≥3					
Clusters		**1**	**5**	**13**	**Mean**	**4**	**7**	**11**	**12**	**Mean**	**p**
n		413	657	189		208	73	762	417		
Demographic data											
Age (years)		60.9±17.0	60.8±16.7	56.9±17.8	60.3±17	56.2±14.7	53.4±17.4	57.1±16.2	56.0±15.9	56.5±16	0.00001
Weight (Kg)		81±25	84.2±26.6	79.3±26.0	82.3±26.0	85.6±29.6	80.7±16.9	84.7±28.1	84.4±28.0	84.5±27.8	0.049
Gender: Females (n/%)		209/50.1	319/48.6	88/46.6	616/49.0	93/44.7	22/30.1	333/43.7	183/43.9	631/43.2	0.003
Mean Charlson-Deyo score		1.0±1.7	1.2±1.9	0.8±1.4	1.1±1.8	0.9±1.6	0.8±1.7	0.9±1.6	0.9±1.7	0.9±1.6	0.025
Number of transplants (kidney and/or liver)		0.09±0.31	0.12±0.38	0.13±0.39	0.096±3.34	0.10±0.36	0.06±0.28	0.05±0.28	0.14±0.40	0.12±0.38	0.049
Clinical/Physiologic											
APACHE III		81±11	88±14	85±12	85±13	102±15	105±14	90±14	95±13	94.4	0.0001
SBP (mmHg)		129±29	126±30	120±29	126±30	113±28	112±30	121±28	112±27	117±29	0.0001
DBP (mmHg)		68±18	66±19	62±19	66±20	57±20	56±21	64±20	59±20	61±20	0.0001
MAP (mmHg)		88±19	86±21	81±20	86±20	75±21	75±22	84±21	77±21	80±21	0.0001
Temperature (^{o}C)		36.8±2.0	36.8±1.3	37.2±1.4	36.9±1.5	36.5±1.3	36.4±1.8	36.8±1.4	36.7±1.5	36.7±1.2	0.0001
Proportion of patients with Temperature < 36^{o}C		0.07	0.11	0.07	0.08±0.02	0.21	0.26	0.15	0.17	0.19±0.05	0.009
DRG (n/%)	Medical	22/73.3	44/62.0	7/53.8	687/54.6	23/53.5	6/31.6	69/59.0	39/54.2	703/48.2	0.003
	Surgical	7/23.3	25/35.2	3/23.1	497/39.5	19/44.2	11/57.9	37/31.6	28/38.9	669/45.8	
	Missing	1/3.4	2/2.8	3/23.1	74/5.9	1/2.3	2/10.5	11/9.4	5/6.9	88/6	
Time to sepsis (minutes)*		272±306	257±294	271±343	264±306	245±308	190±205	227±287	214±266	224±280	0.0001
Lactate (mmol/L)		NA	2.5±2.8	NA	2.5±2.8	5.7±5.11	7.3±5.9	3.1±2.8	4.1±3.9	3.9±3.9	NA

Table 8 Continued

Base deficit (mEq/L)	-0.7±5.4	1.6±6.7	2.0±5.4	1.0±6.3	7.9±7.6	10.6±8.0	3.1±6.2	5.8±6.8	5.0±7.0	0.0001
pH	7.41±0.08	7.36±0.11	7.38±0.09	7.37±0.12	7.28±0.15	7.26±0.15	7.35±0.11	7.32±0.13	7.32±0.13	0.0001
$PaCO_2$ (mmHg)	43.2±15.6	42.6±14.3	38.5±9.7	42.4±14.2	38.0±12.9	34.1±11.2	40.5±13.5	38.2±11.0	39.1±12.7	0.0001
PaO_2 (mmHg)	121±77	135±87	147±81	133±85	155±97	156±86	144±93	151±95	149±94	0.003
SaO_2 (%)	97±4	95±8	97±6	96±6.5	94±14	94±13	96±8	96±7	95±9	0.02
Hemoglobin (mg/dL)	11.1±2.2	11.1±2.2	10.8±2.5	11.0±2.3	10.9±2.7	11.0±3.0	10.8±2.3	10.4±2.4	10.7±2.4	0.001
White blood cell count ($1x10^9$/L)	12.7±6.6	14.9±11.7	13.7±8.2	13.9±8.0	13.8±9.9	18.4±18.21	13.5±8.3	14.6±9.0	14.1±9.5	0.01
Proportion of patients with WBC < $4.5x10^9$/L	0.06	0.05	0.10	0.07±0.03	0.13	0.18	0.10	0.11	0.13±0.04	0.054
Platelet count (1x109/L)	229±122	215±117	202±122	218±120	168±110	174±130	200±123	184±114	190±120	0.0001
Glucose (mg/dL)	161±69	155±65	180±77	160±68	147±70	145±107	164±73	154±71	157±73	0.24
Creatinine (mg/dL)	1.2±1.0	1.2±1.1	1.0±0.4	1.2±1.0	1.1±0.72	1.1±0.9	1.2±0.8	1.1±0.7	1.1±0.8	0.35
Process of care										
Vasopressor use** (n/%)	91/22.0	282/42.9	72/38.1	0.34±0.11	157/75.5	58/79.5	341/44.8	284/68.1	0.67±0.15	0.023
Mechanical ventilation during first 24 hours** (n/%)	177/42.9	474/72.2	121/64.0	771/61.3	184/88.5	70/95.9	571/74.9	355/85.1	1180/80.8	0.00001
RRT ** (n/%)	3/0.7	8/1.2	0/0	11/5.8	9/4.3	3/4.1	8/1.1	9/2.2	29/8.4	0.28
FiO_2	0.41±0.35	0.6±0.3	0.48±0.31	0.54±0.33	0.7±0.3	0.64±0.33	0.61±0.3	0.63±0.3	0.63±0.31	0.0001
Fluids administered*** within first 24 h (L)	1.1±0.61	1.9±0.82	5.8±0.94	2.2±1.7	10.1±1.0	13.9±1.22	4.32±0.71	7.0±0.83	6.4±2.8	0.0001
Outcome data										
ICULOS (days)	9.06±15.4	11.2±16.4	10.2±13.9	10.4±15.7	13.1±14.4	12.2±9.4	12.2±15.7	13.3±15.7	12.7±15.3	0.0001

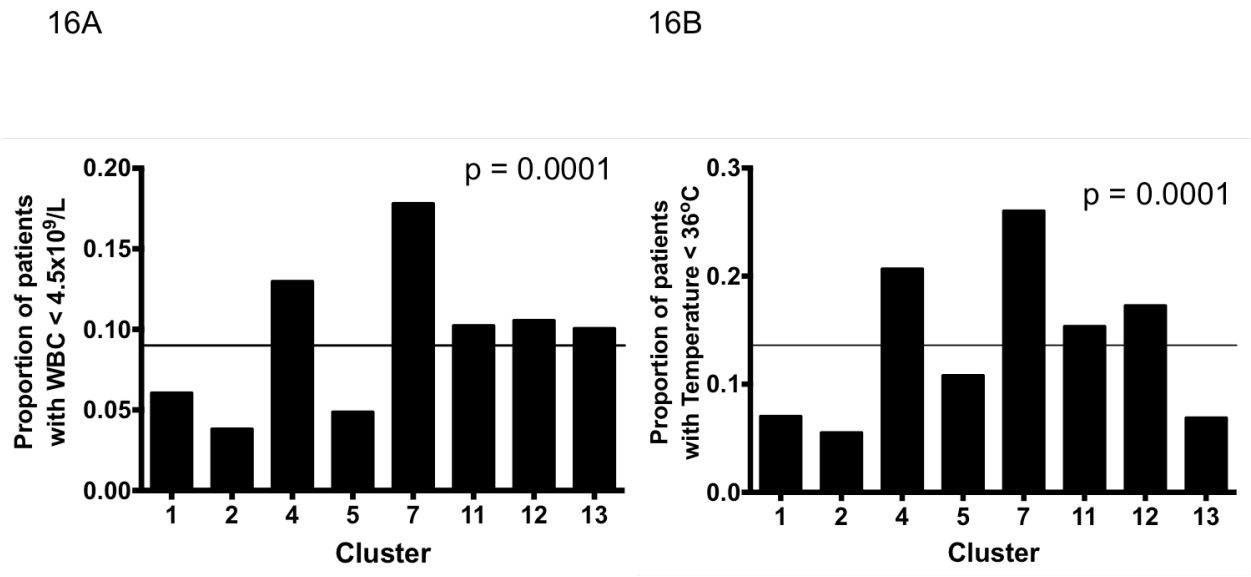
Table 8 Continued

HosLOS (days)	19.8±28.1	22.1±29.8	19.9±20.7	21.0±28.0	21.4±21.9	21.4±18.4	23.7±26.2	24.5±28.0	23.5±25.8	0.016
Mortality 90 days (n/%)	135/32.7	250/38.1	73/38.6	0.36±0.48	88/42.3	36/49.3	262/34.4	171/41.0	0.38±0.49	0.33
Mortality 365 days (n/%)	192/46.5	315/47.9	83/43.9	0.47±0.50	101/48.6	41/56.2	341/44.8	220/52.8	0.48±0.50	0.49

RRT=Renal replacement therapy; *=Time from admission to establishment of diagnosis of "suspected sepsis"; **=at admission.

From a clinical/physiologic standpoint, there were statistical but not clinical differences in systolic, diastolic and mean arterial pressure, although the percentage of patients requiring vasopressor support within the first 24 hours of admission in clusters with 3 or more dysfunctional organs was twice that of clusters with 1-2 compromised organs. There were clear acid base derangements, with higher base deficits in those with more organs compromised, with normal levels of PaCO2, suggesting important metabolic alterations. The proportion of patients with leukopenia (WBC < 4.5 $x10^3$/L) were almost twice as much in the group of clusters with 3 or more dysfunctional organs than in those with 1-2 organs, suggesting important derangements in the immune response. (Figure 16A) In addition, the proportion of patients with hypothermia (defined at temperatures below 36^oC) was more twice as high in the patients with more organs compromised than those with less, again pointing to the observation that these patients may indeed have an alteration in their immune and metabolic response to infection. (Figure 16B) Finally, patients with more organs compromised had lower levels of platelets and a deeper decrement as compared to those with less compromised organs, suggesting perhaps a more important role of coagulation-inflammatory interactions in these patients. From a process of care stand point, patients with more organ dysfunction had more need for mechanical ventilation and

RRT within first 24 hours of admission to the ICU, and required higher amounts of fluid resuscitation as compared to those with less organ dysfunction.



16A. Proportion of patients with admission WBC below $4.5x10^6$/L per cluster. 16B. Proportion of patients with admission temperature below 36^oC per cluster. Black continuous lines represent average proportion of patients for the entire population in each case.

Figure 16. Proportion of patients with leukopenia and hypothermia at ICU admission per cluster.

3.2.3.1 Comparison of clusters characterized by renal, hematologic and hepatobiliary compromise

Clusters 4, 7 and 12 had renal, hematologic and hepatobiliary compromise in addition to cardiovascular and respiratory (group "All"). Clusters 11 and 13 on the other hand had hepatobiliary compromise but not renal or hematologic (group "Hep"). Finally, 1, 2 and 5 had no

renal, hematologic or hepatobiliary compromise (groups "None"). Admission characteristics were compared between these three groups and is shown below in table 9:

Table 9. Descriptive statistics of demographics and admission data stratified by pattern of organ compromise in three groups: All, Hep and None.

Variable	**All**	**Hep**	**None**	**P (ANOVA)**
Clusters	4, 7, 12	11, 13	1, 2, 5	NA
Age (years)	55.7±15.7	57.1±16.2	59.8±17.0	0.0001
Weight (Kg)	84.4±27.6	84.7±28.1	82.2±26.2	0.053
Number of Comorbidities	0.9±1.7	0.9±1.6	1.0±1.7	0.29
Number of transplants (kidney and/or liver)	0.1±0.4	0.1±0.4	0.1±0.3	0.001
APACHE III	98±14	90±14	84±13	0.0001
MAP (mmHg)	76±20	83±21	86±20	0.0001
Temperature (^{o}C)	36.6±1.5	36.8±1.4	37.0±1.5	0.0001
Lactate (mmol/L)	4.9±4.6	3.0±2.8	2.6±2.8	0.0001
Base deficit (mEq/L)	6.9±7.3	3.1±6.2	1.0±6.1	0.0001
White blood cell count ($1x10^{9}$/L)	14.8±10.6	13.5±8.3	13.8±9.2	0.02
Platelet count ($1x10^{9}$/L)	178±115	200±123	222±122	0.0001
Creatinine (mg/dL)	1.1±0.7	1.2±0.8	1.1±1.0	0.63
Fluids administered within first 24 h (mL)	8638±2467	4320±714	2485±1547	0.0001

(See text for further explanation)

The table shows groups based on compromise of renal, hematologic and hepatobiliary compromise (All); only hepatobiliary compromise (Hep); or no compromise of renal, hematologic or hepatobiliary systems (None), aside from cardiovascular and respiratory.

Patients with renal, hematologic and hepatic compromise were younger and had higher APACHE III admission scores. In addition, they had lower admission MAP and temperature, and had metabolic acidosis whereas base deficit for Hep and None was within normal limits. Patients in the "All" group had higher WBC counts than patients in the "Hep" group, but not more than those in the "None" group (Bonferroni). As expected, platelet count was lower in the "All" group as compared to patients in the other groups. However, baseline creatinine was not different between groups. Finally, there were important differences in fluids administered, with higher volumes in the All group, vs. Hep and None.

4.0 DISCUSSION

The aim of the present study was to interrogate a prospectively collected database of critically ill patients selected on the basis of having "suspected sepsis" and evaluate if distinct, clinically sound groups of patients could be found. In doing so, we used clustering analysis which is "the grouping of individuals in a population in order to discover structure in the data"[18]. Given that this study was based on the presumption that the characteristics of such "clusters" of patients if they exist, are completely unknown to us, we selected a method that would allow the data structure to define such the associations between patients. We did this in a two-step approach. First we interrogated the data to find out the amount of "partitions" or clusters this particular data set would have by using Hierarchical clustering. This is the most commonly used method to explore structure in any given data set. It is based on deriving clustering from a given dissimilarity matrix. The dissimilarity between observations (or patients), can be then used to create a Scree plot, and derive from it the number of clusters this particular data set should contain. The second step was to run a K-means algorithm. The aim of the K-means is to partition the data in "k" clusters, so that the within-group sum of squares is minimized. This "k" number of clusters was derived from the Hierarchical cluster analysis. Thus, the investigators had no input on how many clusters should be found, or how the data should be partitioned. This is a simple method that provides objectivity to the derivation of the clusters.

The only intervention where the investigators had input in defining this specific partition of the data came into play when selecting the variables that would fit the hierarchical clustering model. We also tried to objectify this step. We selected the variables by 1. Availability in our data set; and 2. Whether or not it would be feasible to easily collect on admission to the ICU. Then, we ran a correlative matix of all the variables, and found those, which would correlate to each other. If correlation was found, in the setting of biological plausibility, only one of the two variables was selected. Selection between the two was made on the basis of availability in the data (i.e. the variable that was more available in the entire population).

Although any clustering method will yield some sort of partition of the data, regardless of whether it makes sense, we evaluated the obtained clusters for biological plausibility and clinical sense. We found these patients were indeed similar to each other around their clinical characteristics at ICU admission, and thus accepted these groups as valid. The clinical trajectories of the patients suggested later on that clusters could perhaps be further grouped into larger groups. For instance, clusters 4, 7, and 12 were similar between each other, as were 1, 5, and 13. Cluster 11, was sort of in between these two clusters, sharing characteristics of both in different variables, and finally, cluster 2 was definitively different, showing those patients who never had organ compromise at 72 hours or 30 days. In addition, we found that different variables were of different importance when defining cluster membership depending of the cluster. Appendix B1 shows the multinomial logistic regression for cluster membership as well as the coefficients of each variable. These coefficients provide information as to how important each variable in the model is to determine cluster membership. These coefficients represent the impact of the change of 1 unit of the specific variable, in increasing or decreasing (- sign) the

odds of being part of that specific cluster. For example, in the output for cluster 1, the variable with the second largest effect to define membership to cluster 1 was baseline creatinine, with a coefficient of 0.25. This means that for every 1 unit increase in baseline creatinine, the odds of being a member of cluster 1 increase by 0.25. In other words, the higher the patient's creatinine, the higher the likelihood of being in cluster 1. Clusters with 3 or more compromised organs (4, 7, 11, 12), all had use of vasopressors, need for mechanical ventilation and admission temperature as the most important variables defining membership based on their coefficients, whereas clusters 1 and 5 had vasopressor use and baseline creatinine.

When comparing patterns of organ dysfunction, we initially based our analysis on the stipulated published data and definitions of multiple organ dysfunction. By these standards, patients with 2 or more organs should be considered as having multiple organ dysfunction, regardless of how many organs are compromised beyond that definition. However, we found that patients with 2 dysfunctional organs, were more similar with those presenting 1 organ failure, than with those presenting more than 2. We then compared these two groups and clearly found differences, not only in admission demographic and clinical data, but also in the involvement of specific vital signs and laboratory data that could hint towards possible mechanisms of disease. In essence we found that clusters 4, 7, 12 and 11, had a higher proportion of hypothermic, leukopenic and thrombocytopenic patients than clusters 1, 5 and 13, and more so than cluster 2. Although causality cannot be inferred from this study, these data does suggest a potential alteration in the immune, metabolic and endothelial response to injury, which is associated with further organ damage, longer stay and higher 90-day and 1-year death rates.

When we explored the differences between clusters of patients that developed renal, hematologic or hepatobiliary compromise, vs. those who only developed hepatobiliary or none, we found the same signature, with important influence of hypothermia in determining the sickest group. Surprisingly, baseline creatinine did not appear to have an effect on determining the characteristics of these patients or explaining the association with the involvement of these three organs. The strongest predictors of being in the group of renal, hematologic and hepatobiliary compromise (or "All") from the multinomial logistic regression model were use of vasopressors, need for mechanical ventilation and body temperature (Appendix B2). Finally, the administration of fluids in the first 24 hours was clearly distinct between patients with renal, hematologic and hepatobiliary compromise, vs. Hep or None.

This study has important limitations. First, although we attempted to use an unsupervised learning technique, and were careful to objectivize our methodology, it is true that subtle changes in the modeling may cause dramatic changes in the results, and thus this data requires validation and replication before drawing absolute conclusions. In addition, the analysis of this data does not provide use with enough tools as to derive associations between organ dysfunction patterns and mechanisms of disease. This is mainly due to the fact that the data set we used for derivation of this analysis did not include information on other possibly important variables like cytokines, chemokines, specific markers of tissue/organ damage, etc. We expect that this analysis will serve as the basis to explore such mechanistic questions in other databases that will contain such variables. Another limitation of our study is the use of a broad definition of sepsis. We chose to use a broad definition to be able to include all the spectrum of disease in our analysis. However, it is possible that in doing so, we included patients that did not fulfill strict clinical criteria for the

sepsis syndrome, but rather had a localized infection that of course behaves very differently in terms of organ compromise, clinical course and outcome. However, we may have captured most of these patients in cluster 2, and planned analysis will address this question in the future.

5.0 CONCLUSIONS

The present study has demonstrated that an unsupervised clustering technique based on frequently collected demographic, clinical and physiologic data, can be used to interrogate the structure of a large database, and derive distinct and biologically sound clusters of patients. In essence, we have been able to derive specific phenotypes of patients with "suspected sepsis" who look clinically different in some aspects at admission to the ICU, but also, follow distinct trajectories in terms of number of dysfunctional organs, type of dysfunctional organs, length of stay and mortality.

Although the design of the current study does not allow inferring causality, some of the findings suggest potential mechanisms explaining more or less, or even, specific patterns of organ involvement during "suspected sepsis". More leukopenia, hypothermia and thrombocytopenia were related to higher number of compromised organs at 72 hours after admission to the ICU, all of which imply alterations in the immune and endothelial response to injury.

Finally, this methodology provides an interesting tool to define distinct, clinically relevant phenotypes in a cohort of potentially septic patients. It serves as a starting point to select more specific patient populations, and explore diverse mechanisms of disease that may lead to specific

patterns of organ involvement, and may be determine susceptible populations to specific therapeutic strategies in the future.

APPENDIX A: DEFINITION OF COMORBIDITIES AND ORGAN DYSFUNCTION

A1. CHARLSON-DEYO INDEX

Score	Condition
1	Myocardial infarction (history, not ECG changes only)
	Congestive heart failure
	Peripheral vascular disease (includes aortic aneurysm ≥6 cm)
	Cerebrovascular disease: CVA with mild or no residua or TIA
	Dementia
	Chronic pulmonary disease
	Connective tissue disease
	Peptic ulcer disease
	Mild liver disease (without portal hypertension, includes chronic hepatitis)
	Diabetes without end-organ damage (excludes diet-controlled alone)
2	Hemiplegia
	Moderate or severe renal disease
	Diabetes with end-organ damage (retinopathy, neuropathy, nephropathy, or brittle diabetes)
	Tumor without metastases (exclude if >5 y from diagnosis)
	Leukemia (acute or chronic)
	Lymphoma
3	Moderate or severe liver disease
6	Metastatic solid tumor
	AIDS (not just HIV positive)

NOTE. For each decade > 40 years of age, a score of 1 is added to the above score.

Abbreviations: ECG, electrocardiogram; CVA, cerebrovascular accident; TIA, transient ischemic attack; AIDS, acquired immunodeficiency syndrome; HIV, human immunodeficiency virus.

A2. PRE-SPECIFIED RULES TO ASSESS ORGAN FUNCTION

Table A2 shows the criteria we selected to define organ involvement. We adhered to published and validated criteria to define organ dysfunction from different models including the Sequential Organ Failure Assessment score (SOFA), RIFLE criteria for evaluation of AKI, as well as other criteria that represents importance in the clinical setting such as need for vasopressors or need for mechanical ventilation.

Table A2. Criteria to define Organ dysfunction at 72 hours and 30 days

Organ/system involved	Variable	Criteria		Organ dysfunction if:
Cardiovascular	Lactate (mmol/L)	Mild	2-3	72 h and 30 day: Moderate - Severe
		Moderate	3.1-4	
		Severe	>4	
	Use of vasopressors	Low	< Avg	72 h and 30 day: Avg or >Avg
		Average	Avg	
		High	> Avg	
Pulmonary	Mechanical Ventilation	Low	< Avg	72 h and 30 day: Avg or >Avg
		Average	Avg	
		High	> Avg	

Table A2 Continued

	PaO2/FiO2	Mild	200-300	72 h and 30 day: Moderate - Severe
		Moderate	100-200	
		Severe	<100	
Renal	Change in Cr (Based on RIFLE creatinine criteria)	0	< 0.3mg/dL	72 h: R or I 30 day: Persistent elevation > 0.3 mg/dL
		R	> 0.3 mg/dL or 1.5xBaseline	
		I	2xbaseline	
	RRT	Low	< Avg	72h: Avg or >Avg 30 day: doubling 72h rate of use
		Average	Avg	
		High	> Avg	
Hematology	Change in Platelet count	Mild	< 25K	72 h: Severe 30 day: < 150K
		Moderate	25-50K	
		Severe	>50K	
Hepatobiliary	Total Bilirrubin (Based on SOFA criteria)	Mild	1.2-1.9	72 h and 30 day: Moderate - Severe
		Moderate	2-5.9	
		Severe	>6	
	INR	>1.5		72 h and 30 day: >1.5

Avg=Average for the entire population.

APPENDIX B: MULTINOMIAL LOGISTIC REGRESSION

B.1 MULTINOMIAL LOGISTIC REGRESSION FOR CLUSTER MEMBERSHIP

The following is a multinomial logistic regression to assess the determinants of cluster membership. Cluster membership is identified in the analysis as QCL_1. All the variables used in this model were collected either at admission too the ICU (as first measure – i.e. first Temperature), or within the first 24 hours of admission (i.e. need for mechanical ventilation).

```
1 . mlogit QCL_1 last1yr_min_hosp_icu1_MDRD Ap3_1st24hrs_after_ICU1admit age wgt_
  > kg Platelets_1stMeasure_after_ICU1_ WBC_1stMeasure_after_ICU1_admit Temp_1stM
  > easure_after_ICU1_admit i.Sex_code i.vassop_24hrs_from_ICUd1 i.mv_24hrs_from_
  > ICUd1, base(2)

  Iteration 0:   log likelihood =  -6616.165
  Iteration 1:   log likelihood = -6110.1974
  Iteration 2:   log likelihood = -5983.0308
  Iteration 3:   log likelihood = -5969.7557
  Iteration 4:   log likelihood = -5966.3408
  Iteration 5:   log likelihood = -5964.6034
  Iteration 6:   log likelihood = -5964.2893
  Iteration 7:   log likelihood = -5964.2183
  Iteration 8:   log likelihood = -5964.2112
  Iteration 9:   log likelihood = -5964.2097
  Iteration 10:  log likelihood = -5964.2094
  Iteration 11:  log likelihood = -5964.2093
  Iteration 12:  log likelihood = -5964.2093

  Multinomial logistic regression                 Number of obs   =       3315
                                                  LR chi2(120)    =    1303.91
                                                  Prob > chi2     =     0.0000
  Log likelihood = -5964.2093                     Pseudo R2       =     0.0985
```

QCL_1	Coef.	Std. Err.	z	P>|z|	[95% Conf.	Interval]
1						
last1yr_mi~D	.2579879	.0883758	2.92	0.004	.0847746	.4312012
Ap3_1st24h~t	.0016129	.0066398	0.24	0.808	-.011401	.0146268
age	.0078514	.0040984	1.92	0.055	-.0001814	.0158841
wgt_kg	.0000108	.0027442	0.00	0.997	-.0053678	.0053894
Platelets~1_	.0003229	.0005375	0.60	0.548	-.0007306	.0013765
WBC_1stMea~t	-.0135697	.0099723	-1.36	0.174	-.0331149	.0059756
Temp_1stMe~t	-.2179836	.0534588	-4.08	0.000	-.3227609	-.1132063
2.Sex_code	-.2334117	.1380681	-1.69	0.091	-.5040202	.0371969
1.vassop_2~1	.0064545	.1765525	0.04	0.971	-.339582	.3524909
1.mv_24hrs~1	-.4780162	.1360955	-3.51	0.000	-.7447584	-.2112739
_cons	7.408298	2.099799	3.53	0.000	3.292768	11.52383
2	(base outcome)					
3						
last1yr_mi~D	-.994431	.6723972	-1.48	0.139	-2.312305	.3234433
Ap3_1st24h~t	.0884194	.0143552	6.16	0.000	.0602837	.116555
age	-.0364883	.0123909	-2.94	0.003	-.060774	-.0122025
wgt_kg	.0074656	.0061492	1.21	0.225	-.0045867	.0195178
Platelets~1_	-.0035394	.0016921	-2.09	0.036	-.0068557	-.000223
WBC_1stMea~t	.0270984	.0134764	2.01	0.044	.0006852	.0535117
Temp_1stMe~t	-.0449411	.1341549	-0.33	0.738	-.3078799	.2179976
2.Sex_code	.2970106	.432482	0.69	0.492	-.5506385	1.14466
1.vassop_2~1	1.531629	.5423567	2.82	0.005	.4686296	2.594629
1.mv_24hrs~1	17.50022	1929.076	0.01	0.993	-3763.419	3798.419

_cons	-24.60476	1929.083	-0.01	0.990	-3805.538	3756.329
4						
last1yr_mi~D	-.0649433	.1458423	-0.45	0.656	-.3507889	.2209023
Ap3_1st24h~t	.0852312	.0073639	11.57	0.000	.0707981	.0996642
age	-.0197739	.0054445	-3.63	0.000	-.0304448	-.0091029
wgt_kg	.0046003	.003273	1.41	0.160	-.0018147	.0110152
Platelets~1_	-.0048439	.000846	-5.73	0.000	-.006502	-.0031857
WBC_1stMea~t	.007554	.0108196	0.70	0.485	-.0136519	.02876
Temp_1stMe~t	-.200504	.0647012	-3.10	0.002	-.327316	-.073692
2.Sex_code	-.0310781	.1852993	-0.17	0.867	-.394258	.3321017
1.vassop_2~1	1.248666	.2228015	5.60	0.000	.8119829	1.685349
1.mv_24hrs~1	1.225474	.2474216	4.95	0.000	.7405369	1.710412
_cons	-1.023732	2.586609	-0.40	0.692	-6.093393	4.045929
5						
last1yr_mi~D	.2549779	.0842516	3.03	0.002	.0898478	.4201081
Ap3_1st24h~t	.0294701	.0057714	5.11	0.000	.0181584	.0407817
age	.0035735	.003732	0.96	0.338	-.0037411	.0108881
wgt_kg	.0037184	.0023659	1.57	0.116	-.0009187	.0083555
Platelets~1_	-.0014178	.0005103	-2.78	0.005	-.002418	-.0004175
WBC_1stMea~t	.0215602	.0080795	2.67	0.008	.0057246	.0373957
Temp_1stMe~t	-.1935092	.0502	-3.85	0.000	-.2918994	-.0951189
2.Sex_code	-.2377234	.125326	-1.90	0.058	-.4833579	.0079111
1.vassop_2~1	.5098352	.1497216	3.41	0.001	.2163863	.8032841
1.mv_24hrs~1	.5996156	.1290928	4.64	0.000	.3465983	.8526328
_cons	3.688225	1.961814	1.88	0.060	-.1568598	7.53331
6						
last1yr_mi~D	-.7240373	1.922957	-0.38	0.707	-4.492964	3.044889
Ap3_1st24h~t	.006823	.0619719	0.11	0.912	-.1146397	.1282858
age	.0163726	.0519979	0.31	0.753	-.0855414	.1182866
wgt_kg	.0275128	.0183873	1.50	0.135	-.0085257	.0635512
Platelets~1_	.0076743	.0060461	1.27	0.204	-.0041758	.0195244
WBC_1stMea~t	-.1328506	.1369248	-0.97	0.332	-.4012182	.1355171
Temp_1stMe~t	-.6691069	.4119941	-1.62	0.104	-1.476601	.1383867
2.Sex_code	16.11756	2263.238	0.01	0.994	-4419.748	4451.983
1.vassop_2~1	17.16397	2335.924	0.01	0.994	-4561.162	4595.49
1.mv_24hrs~1	14.82352	2198.038	0.01	0.995	-4293.252	4322.899
_cons	-30.47733	3925.613	-0.01	0.994	-7724.538	7663.583
7						
last1yr_mi~D	-.0998418	.2261044	-0.44	0.659	-.5429984	.3433147
Ap3_1st24h~t	.0956655	.0100401	9.53	0.000	.0759872	.1153438
age	-.0317379	.008002	-3.97	0.000	-.0474215	-.0160544
wgt_kg	-.0063246	.005532	-1.14	0.253	-.0171671	.0045179
Platelets~1_	-.0047187	.0012119	-3.89	0.000	-.0070939	-.0023434
WBC_1stMea~t	.0284539	.0105666	2.69	0.007	.0077438	.049164
Temp_1stMe~t	-.1923171	.088448	-2.17	0.030	-.365672	-.0189623
2.Sex_code	.7298071	.2964198	2.46	0.014	.148835	1.310779
1.vassop_2~1	1.277474	.3556348	3.59	0.000	.5804429	1.974506
1.mv_24hrs~1	2.162631	.6065891	3.57	0.000	.9737382	3.351524
_cons	-3.564225	3.609357	-0.99	0.323	-10.63843	3.509984

8						
last1yr_mi~D	-.2773051	1.20194	-0.23	0.818	-2.633064	2.078454
Ap3_1st24h~t	.1487964	.0617574	2.41	0.016	.0277542	.2698387
age	-.0590412	.061134	-0.97	0.334	-.1788616	.0607792
wgt_kg	.0378743	.0194945	1.94	0.052	-.0003342	.0760828
Platelets~1_	-.0302423	.018462	-1.64	0.101	-.0664272	.0059427
WBC_1stMea~t	-.1016881	.1335816	-0.76	0.447	-.3635032	.1601271
Temp_1stMe~t	-.8147279	.5590123	-1.46	0.145	-1.910372	.2809162
2.Sex_code	-.4523989	1.575728	-0.29	0.774	-3.540769	2.635971
1.vassop_2~1	-1.866611	2.136049	-0.87	0.382	-6.05319	2.319967
1.mv_24hrs~1	21.69209	826.2883	0.03	0.979	-1597.803	1641.187
_cons	-5.993347	826.1564	-0.01	0.994	-1625.23	1613.244
9						
last1yr_mi~D	-15.78202	13.90348	-1.14	0.256	-43.03235	11.46831
Ap3_1st24h~t	-.1231165	.1364632	-0.90	0.367	-.3905794	.1443465
age	.0767384	.0956515	0.80	0.422	-.110735	.2642119
wgt_kg	-.1585233	.1066732	-1.49	0.137	-.367599	.0505524
Platelets~1_	-.0215735	.0165433	-1.30	0.192	-.0539978	.0108507
WBC_1stMea~t	-.1201377	.2621024	-0.46	0.647	-.633849	.3935736
Temp_1stMe~t	-1.269956	1.06334	-1.19	0.232	-3.354064	.8141525
2.Sex_code	19.54214	2165.609	0.01	0.993	-4224.973	4264.057
1.vassop_2~1	-13.13544	1560.886	-0.01	0.993	-3072.416	3046.145
1.mv_24hrs~1	15.26587	2374.795	0.01	0.995	-4639.248	4669.779
_cons	38.58915	1921.553	0.02	0.984	-3727.586	3804.764
10						
last1yr_mi~D	-.0337378	.2881904	-0.12	0.907	-.5985806	.531105
Ap3_1st24h~t	.0063434	.0164481	0.39	0.700	-.0258944	.0385812
age	-.0127597	.0101505	-1.26	0.209	-.0326543	.007135
wgt_kg	-.0094978	.0079714	-1.19	0.233	-.0251215	.006126
Platelets~1_	.0013789	.0012511	1.10	0.270	-.0010731	.003831
WBC_1stMea~t	-.0021322	.0238796	-0.09	0.929	-.0489353	.0446709
Temp_1stMe~t	-.2149521	.1346991	-1.60	0.111	-.4789576	.0490533
2.Sex_code	.9225516	.3949488	2.34	0.019	.1484661	1.696637
1.vassop_2~1	.5761773	.415864	1.39	0.166	-.2389013	1.391256
1.mv_24hrs~1	.1687425	.3594363	0.47	0.639	-.5357397	.8732246
_cons	5.162864	5.31817	0.97	0.332	-5.260558	15.58629
11						
last1yr_mi~D	.1373414	.0870959	1.58	0.115	-.0333634	.3080461
Ap3_1st24h~t	.0452444	.0056107	8.06	0.000	.0342477	.0562411
age	-.0115037	.0036077	-3.19	0.001	-.0185746	-.0044328
wgt_kg	.0032525	.0023085	1.41	0.159	-.001272	.007777
Platelets~1_	-.0020228	.0005107	-3.96	0.000	-.0030239	-.0010218
WBC_1stMea~t	.002741	.0083076	0.33	0.741	-.0135415	.0190235
Temp_1stMe~t	-.2143657	.0486574	-4.41	0.000	-.3097324	-.118999
2.Sex_code	-.0309439	.1231993	-0.25	0.802	-.27241	.2105223
1.vassop_2~1	.5006962	.1471652	3.40	0.001	.2122576	.7891348
1.mv_24hrs~1	.6640347	.1275476	5.21	0.000	.4140459	.9140234
_cons	4.503861	1.900447	2.37	0.018	.7790523	8.228669
12						
last1yr_mi~D	.0545929	.1054669	0.52	0.605	-.1521186	.2613043

Ap3_1st24h~t	.0578056	.0063112	9.16	0.000	.0454359	.0701753
age	-.0198908	.0043203	-4.60	0.000	-.0283585	-.0114232
wgt_kg	.0020003	.0027102	0.74	0.460	-.0033116	.0073122
Platelets~1_	-.0038153	.000639	-5.97	0.000	-.0050677	-.002563
WBC_1stMea~t	.0177297	.00887	2.00	0.046	.0003448	.0351146
Temp_1stMe~t	-.1410701	.056615	-2.49	0.013	-.2520334	-.0301068
2.Sex_code	-.021382	.1472752	-0.15	0.885	-.310036	.267272
1.vassop_2~1	1.316855	.1717599	7.67	0.000	.9802121	1.653498
1.mv_24hrs~1	1.126434	.1733581	6.50	0.000	.7866581	1.46621
_cons	.007581	2.225089	0.00	0.997	-4.353513	4.368675
13						
last1yr_mi~D	-.3211752	.181484	-1.77	0.077	-.6768774	.034527
Ap3_1st24h~t	.0190424	.0080497	2.37	0.018	.0032652	.0348196
age	-.009812	.0051427	-1.91	0.056	-.0198915	.0002674
wgt_kg	-.005145	.0036721	-1.40	0.161	-.0123422	.0020522
Platelets~1_	-.0024909	.000761	-3.27	0.001	-.0039824	-.0009994
WBC_1stMea~t	.0147005	.0112979	1.30	0.193	-.007443	.036844
Temp_1stMe~t	.0373748	.072755	0.51	0.607	-.1052225	.1799721
2.Sex_code	-.0112487	.1799187	-0.06	0.950	-.3638828	.3413854
1.vassop_2~1	.6993275	.2074031	3.37	0.001	.2928249	1.10583
1.mv_24hrs~1	.1925977	.1804077	1.07	0.286	-.1609948	.5461902
_cons	-2.67047	2.827676	-0.94	0.345	-8.212614	2.871674

2 .

B.2 MULTINOMIAL LOGISTIC REGRESSION FOR MEMBERSHIP TO "ALL", "HEP" OR "NONE" GROUPS

The following is a multinomial logistic regression to establish the weight of variables defining membership to "ALL", "Hep" or "None" groups. These groups reflect involvement of the renal, hematologic and hepatobiliary system, only the hepatobiliary system but not hematologic or renal, or none, respectively.

```
1 . mlogit  RenHemHepDysf Platelets_1stMeasure_after_ICU1_ Hb_1stMeasure_after_IC
  > U1_admit pH_1stMeasure_after_ICU1_admit  WBC_1stMeasure_after_ICU1_admit Temp
  > _1stMeasure_after_ICU1_admit BD_1stMeasure_after_ICU1_admit i.Sex_code  i.vas
  > sop_24hrs_from_ICUd1 i.vassop_24hrs_from_ICUd1 mv_24hrs_from_ICUd1, base(.)

  Iteration 0:   log likelihood = -914.94822
  Iteration 1:   log likelihood = -818.97132
  Iteration 2:   log likelihood = -818.82466
  Iteration 3:   log likelihood = -818.82464

  Multinomial logistic regression                 Number of obs   =       1320
                                                  LR chi2(9)      =     192.25
                                                  Prob > chi2     =     0.0000
  Log likelihood = -818.82464                     Pseudo R2       =     0.1051

------------------------------------------------------------------------------
RenHemHepD~f |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
1            |
Platelets~1_ |  -.0016792   .0005452    -3.08   0.002    -.0027477   -.0006107
Hb_1stMeas~t |  -.0273172   .0254006    -1.08   0.282    -.0771015    .0224671
pH_1stMeas~t |   1.111628   .7344976     1.51   0.130    -.3279606    2.551217
WBC_1stMea~t |   .0063664   .0068448     0.93   0.352    -.0070492     .019782
Temp_1stMe~t |   .1077261   .0441077     2.44   0.015     .0212767    .1941756
BD_1stMeas~t |   .0922664   .0138995     6.64   0.000      .065024    .1195089
  2.Sex_code |   .1811615   .1223405     1.48   0.139    -.0586216    .4209445
1.vassop_2~1 |   .8596238   .1270366     6.77   0.000     .6106366    1.108611
mv_24hrs_f~1 |   .7739445   .1762279     4.39   0.000     .4285443    1.119345
       _cons |  -13.33266   5.586158    -2.39   0.017    -24.28132   -2.383989
-------------+----------------------------------------------------------------
2            |  (base outcome)
------------------------------------------------------------------------------

2 .
```

BIBLIOGRAPHY

1. Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. *Critical care medicine.* Jul 2001;29(7):1303-1310.
2. Mayr FB, Yende S, Angus DC. Epidemiology of severe sepsis. *Virulence.* Jan 1 2014;5(1):4-11.
3. Angus DC. The lingering consequences of sepsis: a hidden public health disaster? *JAMA : the Journal of the American Medical Association.* Oct 27 2010;304(16):1833-1834.
4. Dellinger RP, Levy MM, Rhodes A, et al. Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock: 2012. *Critical care medicine.* Feb 2013;41(2):580-637.
5. Majno G. The ancient riddle of sigma eta psi iota sigma (sepsis). *The Journal of infectious diseases.* May 1991;163(5):937-945.
6. Marshall JC. Sepsis: rethinking the approach to clinical research. *Journal of leukocyte biology.* Mar 2008;83(3):471-482.
7. Samraj RS, Zingarelli B, Wong HR. Role of biomarkers in sepsis care. *Shock.* Nov 2013;40(5):358-365.
8. Ranieri VM, Thompson BT, Barie PS, et al. Drotrecogin alfa (activated) in adults with septic shock. *The New England journal of medicine.* May 31 2012;366(22):2055-2064.
9. Bernard GR, Vincent JL, Laterre PF, et al. Efficacy and safety of recombinant human activated protein C for severe sepsis. *The New England journal of medicine.* Mar 8 2001;344(10):699-709.
10. Reinhart K, Menges T, Gardlund B, et al. Randomized, placebo-controlled trial of the anti-tumor necrosis factor antibody fragment afelimomab in hyperinflammatory response during severe sepsis: The RAMSES Study. *Critical care medicine.* Apr 2001;29(4):765-769.
11. Charlson M, Szatrowski TP, Peterson J, Gold J. Validation of a combined comorbidity index. *Journal of clinical epidemiology.* Nov 1994;47(11):1245-1251.
12. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *Journal of chronic diseases.* 1987;40(5):373-383.
13. Vincent JL, Moreno R, Takala J, et al. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. *Intensive care medicine.* Jul 1996;22(7):707-710.

14. Mistichelli J. Diagnosis Related Groups (DRG) and the Prospective Payment System: Forecasting Social Implications. https://bioethics.georgetown.edu/publications/scopenotes/sn4.pdf. Accessed 05/03, 2014.
15. Knaus WA, Wagner DP, Draper EA, et al. The APACHE III prognostic system. Risk prediction of hospital mortality for critically ill hospitalized adults. *Chest.* Dec 1991;100(6):1619-1636.
16. Force ADT, Ranieri VM, Rubenfeld GD, et al. Acute respiratory distress syndrome: the Berlin Definition. *JAMA : the journal of the American Medical Association.* Jun 20 2012;307(23):2526-2533.
17. Srisawat N, Kellum JA. Acute kidney injury: definition, epidemiology, and outcome. *Current opinion in critical care.* Dec 2011;17(6):548-555.
18. Webb AR, Copsey KD. *Statistical Pattern Recognition.* Third ed. Chichester, United Kingdom: Wiley; 2011.